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LIST OF ABBREVIATIONS

AA: Arachidonic acid
ALP: Alkaline phosphatase
ALT: Alanine transaminase
AP1: Totally hydrolyzed FB1
AST: Aspartate aminotransferase
AUC: Area under plasma concentration-time curve
B.W: Body Weight
BSA : Bovine serum albumin
C max : Maximum plasma concentration
C1P : Ceramide-1-Phosphate
CAT : Catalase
Cer : Ceramide
CerS: Ceramide synthase
CHOL: Cholesterol
CK: Creatinine kinase
Cl: Total plasma clearance
CV: Coefficient of variation
DHCD: Dihydroceramide desaturase
ELEM: Equine leuko-encephalomalacia
EU: European Union
F: Bioavailability
FB1: Fumonisin B1
FB2: Fumonisin B2
FB3: Fumonisin B3
FBs: Fumonisin
FDA: Food and Drug Administration
GGT: Gamma Glutamyl Transferase
GSH: Glutathione
H₂O₂: Hydrogen peroxide
HBF1/2: partially hydrolyzed FB1
HCl: hydrogen chloride
HDL: High-density lipoprotein

HPLC: High-performance liquid chromatography
HPLC-MS: High performance liquid chromatography mass spectrometry
IARC: International Agency for Research on Cancer
IFNs: Interferons
IL: Interleukin
IP: Intraperitoneal
IV: Intravenous
JECFA: Joint FAO/WHO Expert Committee on Food Additives
LDH: Lactate Dehydrogenase
LDL: Low-density lipoprotein
LOD: Limit of Detection
LOEL: lowest-observed-effect-level
LOQ: Limit of Quantification
MRT: Mean Residence Time
NOEL: No -observed-effect-level
OPA: Ortho-Phtalaldehyde
PBS: Phosphate-Buffered Saline
PG2 α : prostaglandin 2 alpha
PKC: Protein Kinase C
PMTDI: Provisional Maximum Tolerable Daily Intake
PPE: Porcine pulmonary edema
R²: coefficient of determination
RBC: Red Blood Cell
Sa C20: C20 Sphinganine
Sa: So ratio: Sphinganine to Sphingosine ratio
Sa: Sphinganine
Sa-1P : Sphinganine-1-Phosphate
SAX: Strong Anion Exchange
SMS: Sphingomyelin synthase
So: Sphingosine
So-1P: Sphingosine-1-Phosphate
SPT: Serine Palmitoyl Transferase
ST: Stimulate
T 1/2 β : Elimination half-life

T_{1/2α}: Distribution half life

TLC: Thin layer chromatography

T_{max}: Time of maximum plasma concentration

TNF-α: Tumour Necrosis Factor-α

TP : Total Protein

UV : Ultra Violet

V_c: Volume of the central compartment

V_{darea}: Volume of distribution method of area

V_{dss}: Volume of distribution at the steady state

VLDL: Very-low-density lipoprotein

[¹⁴C] FB1: Radiolabelled Fumonisin B1

3KSR: 3-Keto-Sphinganine Reductase

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Motivation of PhD

I decide to choose this subject for three principle reasons:

1- Researcher reasons: I am researcher in Biotechnology Research Center in Libya. In my job in Libya, we have all the necessary requirements of laboratory equipments, but we do not have enough experience to work on it. Then, this subject can improve my knowledge and experience in laboratory equipments, particular in HPLC.

2- Animal health reasons: I am veterinarian and oversee a number of poultry farms in Libya. The poultry production is the principle animal production in Libya. However, this sector suffering from several health problems which leads to huge mortality, and economic disasters. This subject permits me to investigate the adverse effect of fumonisins on poultry production (mortality, and body weight), and health (toxicity, and immunological reaction).

3- Human health reasons: veterinary medicine is the first defense line of human health. Therefore, this subject has contributed to increase my knowledge about extraction and/or residual of toxic molecules from cereals and animal products. Therefore this subject helps to introduce health food for human consumption.

RESUME

Les fumonisines (FBs) sont les principales mycotoxines produites par *Fusarium verticillioides* et *Fusarium proliferatum*, qui se retrouvent partout dans le monde dans le maïs et ses produits dérivés. Les doses toxiques et les signes cliniques de toxicité provoqués par les FBs varient d'une espèce à l'autre. La toxicité des FBs est généralement liée à leur capacité à bloquer le métabolisme des sphingolipides chez les espèces animales, y compris chez les espèces aviaires. De précédentes études ont démontré que les canards présentent une plus grande sensibilité à la toxicité des FBs que les dindes, alors que l'accumulation de sphinganine (Sa) dans les tissus est plus importante chez les dindes que chez les canards.

L'objectif de nos travaux était de comprendre les différences de toxicité entre les dindes et les canards lors d'une exposition aux FBs. Les trois hypothèses suivantes ont été explorées : i) La toxicocinétique de la fumonisine B2 chez les dindes et les canards. ii) La capacité des cellules aviaires à se protéger de l'importante accumulation de sphingolipides libres en augmentant leur catabolisme (phosphorylation). iii) Des mécanismes de toxicité des FBs autre que leur altération via le métabolisme des sphingolipides (stress oxydatif et les réponses inflammatoires).

L'analyse des paramètres de toxicocinétique de la fumonisine B2 n'a pas mis en évidence de différence significative entre les dindes et les canards. Les mesures de la toxicité simultanée de plusieurs FBs chez les dindes et les canards ont confirmé la forte sensibilité des canards. L'accumulation de shingasine-1-phosphate (Sa1P) dans le foie a également été corrélée avec la quantité de Sa mais pas avec les paramètres hépatiques de toxicité. De plus cette étude a mis en évidence que la quantité de Sa dans le foie était fortement dépendante de la teneur en FBs. Cependant les FBs n'ont eu aucun effet sur les paramètres de stress oxydatif pour les deux espèces. De manière intéressante, les FBs ont eu une légère réponse inflammatoire chez les canards mais pas chez les dindes. Des investigations plus poussées sur les effets des FBs sur le métabolisme des céramides et sur les processus inflammatoires seraient nécessaires pour comprendre les différences de toxicité entre les dindes et les canards exposés aux FBs.

SUMMARY

Fumonisin (FBs) are the major mycotoxins produced by *Fusarium verticillioides* and *Fusarium proliferatum*, which are found worldwide in maize and maize products. FBs toxic dose and clinical signs of toxicity vary from one species to another. FBs toxicity is commonly linked to their ability on blocking sphingolipids metabolism in all animal species, including avian species. Previous studies have demonstrated that ducks exhibit higher sensitivity to FBs toxicity than turkeys, whereas, the accumulation of sphinganine (Sa) in tissues is more pronounced in turkeys than in ducks.

The objectives of our works were to investigate the causes which lead to different toxicity between ducks and turkeys to FBs exposure. The following three hypotheses were investigated: i) Toxicokinetics of fumonisin B2 in ducks and turkeys. ii) Ability of bird cells to protect themselves against high accumulation of free sphingolipids by increasing their catabolism (phosphorylation). iii) Other toxicity mechanisms of FBs rather than their alteration of sphingolipids metabolism (oxidative stress damage and inflammatory responses).

The analysis of toxicokinetic parameters of fumonisin B2 did not provide a significant difference between ducks and turkeys. The measurement of simultaneous toxicity of FBs in ducks and turkeys confirmed higher sensibility of ducks. Also the accumulation of Sphinganine-1-Phosphate (Sa1P) in the liver correlated with the amount of Sa but not parameters of hepatic toxicity. Moreover, this study revealed that the amount of Sa in the liver was strongly dependent on the amount of FBs. On the other hand, FBs had no effect on oxidative damages parameters in both species. Interestingly, FBs had mild inflammatory response effect in ducks but not in turkeys. Further investigation on the effects of FBs on ceramide metabolism and inflammatory processes would be necessary to understand the different toxicity between ducks and turkeys to FBs exposure.

INTRODUCTION

Fumonisin (FBs) are the most important mycotoxins produced by *Fusarium verticillioides* and *Fusarium proliferatum* fungi, which are widely found as contaminants in corn and corn screenings [48]. Although a number of different FBs have been isolated from culture, the most common is fumonisin B1 (FB1), with lesser amounts of fumonisins B2 (FB2) and B3 (FB3) being known to naturally occur [1-18-21-150]. Many researchers considered that FBs have a strong relation to certain diseases in different animal species such as encephalomalacia in horses [9], pulmonary edema pigs [11-12], and hepatic and renal toxicities in equines, pigs, sheep, rodents and poultry [15-16-17]. Additionally, FB1 is considered as a cancer initiator and a strong cancer promoter for hepatocarcinoma in rats [10-94]. The carcinogenic properties of FB1 have been confirmed by the National Toxicology Program (NTP) study, which demonstrated FB1 to be nephrocarcinogenic in male rats and hepatocarcinogenic in female mice [207]. Furthermore, FB1 had implicated in the high incidence of human esophageal cancer in South Africa and China [4-21-77], and it was considered a primary risk factor for human liver cancer in China [77-273]. Currently, all animal species appear to be sensitive to FB1 exposure, but its toxicity differs from one species to another. In short term, carcinogenesis studies on rats have indicated that fumonisin B2 (FB2) and FB3 have similar effects to FB1 in terms of toxicity and hepatocarcinogenicity [48]. IARC (International Agency for Research on Cancer) classified FB1 and FB2 as class 2B derived carcinogenic [267]. In order to solve these problems, several recommendations and regulations have been made by the FDA, JECFA and EU to limit FB1, FB2 and FB3 in food consumed by humans and animals [25-157-160-267-289]. For example, in France mycotoxin inspections showed that 67% of corn samples were contaminated by *Fusarium verticillioides* and that more than 80% of these strains were able to produce very high levels of FB1 in laboratory conditions [276]. Moreover, around 10% of corn samples analyzed by the Toxicological group (department) at the Veterinary school of Toulouse were contaminated at levels between 10 and 20 mg of FB1/kg [158]. Very high levels of FB1 (100 to 200 mg/kg) were recorded in the feed of horses suffering from ELEM in Toulouse [105].

Actually, there is little data available concerning fumonisins in poultry which consume high quantities of corn during their lives particularly in France where ducks consume about 1Kg of corn per day during the force-feeding program to produce fatty liver [158]. All those facts pushed the Toxicological group (Department) at the Veterinary school of Toulouse to induce several researches to investigate the effects of fumonisins and their consequences on poultry. The results obtained from those researches demonstrated that ducks were more sensitive to FB1 toxicity than turkeys [156-158-165]. By contrast, absorption and persistence of FB1 were higher in turkeys than in ducks, and excretion of FB1 is lower in turkeys than in ducks [23-24].

The objective of the PhD is to investigate the causes which lead to different toxicity between ducks and turkeys to FBs exposure. Two experiments (FB2-Toxicokinetics and FBs-Toxicity) on ducks and turkeys at same time were conducted in order to explain the aim of the PhD.

CHAPTER 1: LITERATURE REVIEW

I. Fumonisin general introduction

1. Historical background of fumonisins

In 1900, fumonisin toxic effects were observed for the first time after sporadic fatal conditions in horses in countries such as the United States, China, Japan, Europe, South Africa and Egypt [18-1]. In 1902, Mr. Butler named the disease equine leukoencephalomalacia (ELEM) after inducing its symptoms in tested horses fed with moldy feed. Other names used to describe it were blind staggers, foraging disease, moldy corn poisoning, leukoencephalitis, and cerebritis [2-3].

In 1970, an outbreak of ELEM in horses in South Africa was associated with the contamination of corn by the fungus *Fusarium verticillioides* in certain areas [19].

In 1971, Wilson confirmed that causative agents of ELEM are maize and cereals infected with genus *Fusarium* mold. In particular, *Fusarium moniliforme* was implicated [4]. Nevertheless, this explanation was not very precise, because *Fusarium moniliforme* could produce a range of mycotoxins, including trichothecenes, zearalenone, fusaric acid, moniliformin, fusarin C, and the fumonisins [5].

In 1988, the real causative agent of ELEM in South Africa was discovered by Marasas's group (Program on Mycotoxins and Experimental Carcinogenesis-PROMECC), when the two toxic metabolites (FB1 and FB2) were isolated from contaminated maize with *Fusarium verticillioides* (synonym *Fusarium moniliforme*) [1-18-21].

In 1990, Kellerman observed typical symptoms of ELEM after horses were exposed to purified FB1 by oral route [9]. Since then numerous studies have been performed to better understand the adverse effects of FB1 on different animal species. The results obtained from those studies confirmed that FB1 was implicated in hepatic and renal toxicities in equines, pigs, sheep, rodents and poultry [15-16-17], pulmonary oedema in pigs [11-12], and liver cancer in rats [10]. FB1 had not shown to cause esophageal cancer in all tested animal species [13-14]. However, high incidences of esophageal cancer were observed in 1970, in Transkei - South Africa among people who ate homegrown corn [4-21]. In addition, in 1988, Marasas and his group conducted a fungus isolated comparative study between esophageal cancer

areas and non-esophageal cancer areas. The main type of fungus isolated from infected areas was *Fusarium verticillioides* [7-20-21].

2. Chemical and physical characteristics of fumonisins

Fumonisin B1 is the most widespread type of FBs [1-18-21-150]. Chemical formula of FB1 is $C_{34}H_{59}NO_{15}$, and it is the diester of propane-1,2,3-tricarboxylic acid and 2-amino-12,16-dimethyl-3,5,10,14,15-pentahydroxyeicosane (molecular weight: 721), (figure 1, and table 1) [18]. The pure substance of FB1 is a white hygroscopic powder which is soluble in water, acetonitrile-water or methanol-water. It is stable in acetonitrile-water (1:1), food-processing temperature and light. FB1 is unstable in methanol [18].

In nature, there are about 15 different structures of fumonisin which are classified to four groups (A, B, C and P). Each group is divided in subclasses as FA1, FA2, FA3, FAK1, FB1, FB2, FB3, FB4, FC1, FC2, FC3, FC4, FP1, FP2 and FP3 [18-21-167].

The B class fumonisins are esters of 2-amino-12, 16-dimethyl- 14, 15-dihydroxyeicosane, and propan-1, 2, 3-tricarboxylic acid. FA1, FA2 and FA3 are N-acetyl derivatives of FB1, FB2 and FB3. FAK1 is like FA1. FCs differs from other fumonisins by lack of a methyl group. FPs have 3-hydroxypyridium group instead of the amine group in the FBs (figure 1, table 1) [18].

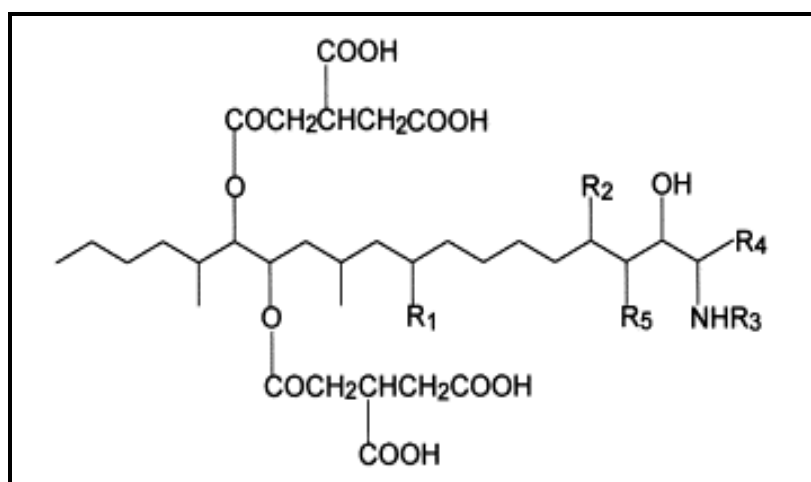


Figure 1: Chemical Structure of FBs [21-167]

Table 1: Classification of fumonisins [167]

Fumonisin	R1	R2	R3	R4	R5
FA1	OH	OH	CH ₂ CO	CH ₃	H
FA2	H	OH	CH ₂ CO	CH ₃	H
FB1	OH	OH	H	CH ₃	H
FB2	H	OH	H	CH ₃	H
FB3	OH	H	H	CH ₃	H
FC1	OH	OH	H	H	H

3. Occurrence of fumonisins

Incidence of cereals contamination by *Fusarium moniliforme* increased in hot-humid regions, and it is related to insect invasions [81]. Maize, compared to other cereals, is most frequently contaminated by fumonisins. In addition, fumonisins have been found at high level in wheat, asparagus, tea, and cowpea [150]. In fact, it is very difficult to obtain uncontaminated maize, even if the contamination level is not significant. Fumonisin has been detected in cereals at very low concentration as 0.02 mg/kg (it is the limit of detection in many investigations), but sometimes they reach up to tens of mg/kg [150]. In most samples of investigation, FB1 is the most prevalent toxin, with co-occurrence of FB2 and FB3 [18-150]. Several countries in Africa, North- and South America, Asia, and Europe have reported FB1 in cereals at levels from 0.02 to 25.9 mg/kg, and FB2 at levels from 0.05 to 11.3 mg/kg [224]. However, quantity of fumonisins in animal feed is reported in South Africa at level from 4.0 to 11.0 mg FB1/kg [225], in Uruguay from 0.2 to 6.3 mg FB1/kg [226], and in France up to 2.1 mg FB1/kg and 0.9 mg FB2/kg [227]. The studies conducted on dry milling maize reported that the highest level of FB1 occurred in germ, bran and animal feed flour, respectively. Also FB1 is present at higher levels in small corn grits compared to large corn grits and human food flour [228-229-230]. Therefore, preparation of human and animal food from cereal fractions (germ alone or bran alone) could contain higher levels of FB1 than the feed made by raw materials. In addition, there is some evidence that maize silage contains low levels of FB1 (around 0.6 mg FB1/kg) [231].

II. Fumonisin toxicokinetic

Toxicokinetic is the study of the rate of a chemical in the body and what happens to it after it reached the plasma. These studies are divided in four steps called absorption, distribution, metabolism and elimination (ADME) [70]:

- Absorption, measured by the bioavailability, represents the amount of the administered compound that reaches the plasma and the kinetic of its appearance in this fluid. The absorption reveals the relation between dose of exposure and systemic exposure of the body. It permits to extrapolate parenteral and oral exposure, to reveal differences between species, and, in a lesser extent, to predict risk of persistence in the body (risk of residues).
- Distribution, measured by the volume of distribution, represents the compartments of the body where the toxin reaches. It could explain toxicity (accumulation in a tissue), and is linked with the ability of a toxic to be metabolized (see below). A large distribution often goes with high systemic exposure to a toxic whereas a low one is generally observed for compound that known a rapid excretion (see below).
- Metabolism represents the biochemical transformation of a toxic by systems known as "drug metabolizing enzyme". These transformations can increase or decrease toxicity. Strong variations have been observed between species concerning their ability to metabolize a compound. These differences are often involved to explain interspecies differences of toxicity.
- Elimination, measured by the clearance, represents the output of a compound from the body. Different mechanisms are involved: metabolism into a new compound, excretion in body fluid (urine, bile, milk...). High clearance of a compound represents a rapid disappearance of this compound from the body. It generally goes with a low ability to accumulate. By contrast, a low clearance means that the compound accumulates within the body, which has consequences in terms of toxicity (chronic exposure) and persistence of residual.

The ADME parameters are obtained after administration of the compound by oral and parenteral route and monitoring of the plasma concentrations. Sometimes,

direct measurements of the toxic within the tissues are available. Metabolizing studies can also be performed *in vivo* or *in vitro* (cell cultures) by using purified drug metabolizing enzymes. Usually, the kinetic of the toxic in the plasma is modeled by using one, two or three exponential. The calculation of the toxicokinetic parameters and their unit are described in the materials and methods section of this document for the toxicokinetic of FB2 (page 89). In this chapter, the toxicokinetic parameters of FBs are presented to reveal differences between species that could explain differences of toxicity and, when available, differences between fumonisins (FB1, FB2 and FB3). A specific focus will be done on the avian species.

Fumonisin toxicokinetic and metabolism data for humans are not available. Many efforts have been made to understand the kinetics and metabolism of FB1 in different animal species like cows, pigs, rats and poultry. In the past, global research was conducted by radiolabelled [¹⁴C] FB1 [18]. Whereas recently, a new method was developed to determine non-radio-labelled FB1 (unlabelled) in serum by using strong anion exchange (SAX) cartridges to extract fumonisin, OPA (ortho-phthalaldehyde) to give fluorescent properties to FB1, and HPLC to quantify FB1 [22-23-24-32]. By contrast, the kinetics and metabolism of FB2 have been very poorly documented. The first study describing toxicokinetics of FB2 in rats was done in 1995. And a second one was conducted on non-human primates in 1999. These studies were carried out by Shephard [28-51]. In addition, data related to pharmacokinetics of fumonisin hydrolyzed forms in all animals species, and FB2 in avian are not available.

1. Absorption

Until recent times, there has been no evidence of absorption of fumonisins by inhalation or skin contact. However, natural presence of fumonisins in *F. verticillioides* cells (mycelia, spores and conidiophores) increase the possibility of absorption by inhalation or buccal exposure. FBs are water soluble and polar compounds. Thus their ability to penetrate an intact skin is not strong. Although it may be stronger if the skin is damaged [18].

In general, all tested animal species confirmed that FB1 has poor absorption ability (less %5 of dose), with approximately T_{max} from 3 to 5 hr, as synthesized in table 2.

Table 2: Absorption of fumonisins in different animal species

Animals	Dose ¹ FB1 (mg/kg b.w)	T_{max} (hr)	C_{max} (µg/ml)	Bioavailability (% of dose)	Ref
Rats	10	1	0,18	3,5	[32]
Monkeys	6,42*	1-2	< 0.21	2	[35]
Pigs	0,5*	1,10	0.033	4	[31]
	5	2	282	NC	[63]
Cattle	1 or 5	ND	ND	0	[34]
Layer hens	2*	1,5-2,5	0.028-0.103	0.7	[30]
Turkeys	100	3	0.991	3.2	[24]
Ducks	100	1	0.559	2.1	[23]
Animals	Dose ¹ FB2 (mg/kg b.w),	T_{max} (hr)	C_{max} (µg/ml)	Bioavailability (% of dose)	Ref
Rats	7,5	ND	ND	ND	[28]
Monkeys	7,5	3-5	0.025-0.04	NC	[51]

¹ single oral dose of FB1 or *[14C] FB1 or FB2

C_{max}: maximum serum concentration; T_{max}: time of the C_{max}; NC: not conducted; ND: non detected.

The absorption of radiolabelled [14C] FB1 is fairly close in avian and mammalian species, 0.71% and 2-4% of dose, respectively [30-31-35]. Quite similar results were announced by administering different dosage of unlabelled FB1 via oral route to rats, ducks and turkeys. The results concluded that avian and mammalian species have similar bioavailability. It is 2.1-3.2 and 3.5 % of administered dose, respectively, (table 2). Overall, the rates of bioavailability between avian species and mammals are close to each other [23-24-32-51].

Analogous bioavailability can be found in avian species by using little single dose 2 mg/kg b.w. of radiolabelled FB1 for laying hens, or by using high single dose 100 mg/kg b.w. of unlabelled FB1 for ducks and turkeys. Those experiments demonstrated that maximum absorption time was between 60 to 180 min. And the bioavailability was 0.71%, 2.3% and 3.2% respectively (table 2) [23-24].

In literature, there is data clarifying that bioavailability of FB1 is poor in ruminants compared to that obtained in monogastric animals. FB1 is unable to being recovered in serum after single oral dose of 1 or 5 mg FB1/kg b.w. [34]. Rice and Ross (1994) demonstrated that lower FB1 absorption in cattle and sheep is related to rumen micro-flora metabolic activity, which converts FB1 to hydrolyzed forms

(HFB1). This explanation is given after exposing cattle to 50-400 mg FB1/kg of feed, for one month. The results obtained in this experiment showed that 60-90% of total FB1 recovered in feces was in hydrolyzed form HFB1. It is believed that rumen metabolism could interfere with FBs absorption, which reaches less than 1% of the dose [18-36]. By contrast, Smith and Thakur (1996) mentioned that more than 80% of fumonisins (FB1 and FB2) in ruminant feces is unmetabolized or unhydrolyzed. This result was obtained by feeding steers with 400 mg FB1/kg and 130 mg FB2/kg of feed for one month [52].

It has been demonstrated that absorption of HFB1 in rats is higher than FB1 by two folds, but it was not significant. This experiment does not depend on a toxicokinetic study, but rather on excretion percentage of molecules from the urine after gavage of single dose 0.69 μ mol of 14C- FB1 and 14C- HFB1/kg b.w. [71].

Lack of detection of FB2 in the plasma at any time after oral administration of 7.5 mg FB2/kg b.w. in rats and monkeys, shows that bioavailability of FB2 is as limited as the one of FB1. Even if trace levels of FB2 was detected in plasma after an oral exposure dose in monkeys. That means bioavailability of FB2 is still less important than bioavailability of FB1 in mammals [28-51].

2. Distribution

Fumonisin B1 has a rapid distribution, with a distribution half-life ($T_{1/2\alpha}$) less than 3 min in all tested animals [23-24-30-32-34-53].

In avian species, the same time of distribution half life of FB1 ($T_{1/2\alpha}$) is recorded in laying hens and ducks (2.5 and 2.6 min, respectively), by using different types of FB1 (radiolabel and unlabelled) [23-30]. On the other hand, equal distribution half life of FB1 ($T_{1/2\alpha}$) is obtained (1.7 min) in different species of turkeys and cattle by using the same type of FB1 (unlabelled FB1) [24-34]. All those results indicate that the distribution half-life of FB1 is equal in the same species by using different types of FB1 (radiolabel and unlabelled). Also, the same type of FB1 (unlabelled FB1) has same distribution half life ($T_{1/2\alpha}$) in different species (table 3).

Table 3: Distribution of FB1 in different animal species after IV dosing

Animals	Dose ¹	Exponential	Parameters	Ref
Rats	2	Two	Vd area: 0.11 L/kg	[32]
Pigs	0.4*	Three	T1/2 α : 2.2 min Vd area: 2.41 L/kg	[31]
Cow	0.05 – 0.2	Two	T1/2 α : 1.7 min. Vd area : 0.26 L/kg Vc 0.05 L/kg	[34]
Laying Hens	2*	Two	T1/2 α : 2.5 min Vd area: 0.08 L/kg Vc : 0.005 L/kg	[30]
Turkeys	10	Three	T1/2 α : 1.7 min Vd area: 1 L/kg Vc: 0.1 L/kg	[24]
Ducks	10	Two	T1/2 α : 2.6 min Vd area: 0.8 L/kg Vc: 0.2 L/kg	[23]

¹ single IV dose (mg/kg b.w.) of FB1 or *[14C] FB1;

T1/2 α : distribution half-life; Vd area: volume of distribution; Vc: volume of central compartment.

The distribution of radiolabelled FB1 in laying hens is less important than the distribution of unlabelled FB1 in ducks and turkeys. This description depends on the volume of distribution (Vd-area) in laying hens, ducks and turkeys (Vd area: 0.08, 0.8 and 1 L/kg, respectively). That means ducks and turkeys are more susceptible to residual FB1 in their tissues than laying hens [23-24-30]. The difference in the volume of distribution (Vd-area) in avian species is not related to the difference in the administrated types of FB1 (radiolabel or unlabelled), but it is rather related to avian classification. That point was proven by the volume of distribution of FB1 obtained in rodents which was quite similar to that of laying hens (Vd (area) 0.11 and 0.08 L/kg, respectively), even though they were different species exposed to different types of FB1 (table 3) [30-32].

In rodents, Vd-area was 0.11 L/kg in a rat injected with a single dose of 2 mg FB1/kg b.w., by IV route [32]. In pregnant rats, one hour after administration of 101 mg [14C- FB1]/kg b.w. by intravenous, around 14.5% and 4% of radiolabelled FB1 is distributed to the liver and kidneys, respectively (table 3). Moreover, only a trace element is recovered in the uterus 0.24% to 0.44%, placenta 0 to 0.04% and total fetus

recovery < 0.015% of dose. These results signified that FB1 is incapable of crossing rat placenta, (table 3) [53].

In pigs, FB1 shows higher distributing ability through their bodies when compared to avian or mammalian species. Those results were obtained after pigs were intravenously exposed to a single dose of 0.4 mg [¹⁴C-FB1]/kg b.w. The data obtained established that distribution half life ($T_{1/2 \alpha}$) was 2.2 min. In addition, the volume of distribution (V_d area) is 2.41 L/kg [31].

The data collected from lactating dairy cows confirmed that the distribution of FB1 does not depend on the quantity of the dose exposure or the animal species. That information was collected after exposing cows to low and high intravenous doses of 0.05 or 0.2 mg of FB1/kg b.w. Their results demonstrated that $T_{1/2 \alpha}$ in both concentrations was 1.7 min. Volume of distribution (V_d area) in low and high concentration doses were 0.251 and 0.278 L/kg, respectively. During that time, the volume of central compartment (V_c) was 0.054 and 0.046 L/kg, respectively [34]. Same number of distribution half life ($T_{1/2 \alpha}$) was obtained in cattle and turkeys [24-34].

Unfortunately, data concerning distribution of FB2 are not available. All those researches are simplified in table 3, to give idea about FB1 distribution in different animal species in comparison with avian species.

3. Metabolism

The liver is generally the principal site to xenobiotic metabolism, but occasionally it can take place in the gastrointestinal tract, lungs, kidneys, or serum. Moreover, xenobiotic can be degraded by intestinal microflora to less or higher active molecules [67-70].

Fumonisin B1 molecule consists of long chain aminopentol backbone (AP1), with two ester-linked to tricarballic acids (TCA). FB1 that lack TCA at one side of the chain replaced by hydroxyl group (OH) are called partially hydrolyzed (HFB1/2) or aminopolyols (AP1/2). Concurrently, they are called fully hydrolyzed (AP1) when the lack of TCA occurs at both sides of the chain [18-57-68-74].

Vervet monkeys were exposed to a single dose of 1.72 mg (intravenous) and 42 mg (gavage) [¹⁴C- FB1] / kg b.w. The results obtained from gut contents and feces

after IV exposure demonstrated that 7.5%, 33% and 1.5% of the dose was recovered as unhydrolyzed, partially hydrolyzed and full hydrolysis form (aminopentol-AP1), respectively. Concurrently, the results obtained by oral route demonstrated that 48%, 13.5% and <0.1% of the dose were recovered as unhydrolyzed, partially hydrolyzed and full hydrolyzed, respectively. Overall, in both ways of toxin administrations no hydrolyzed forms in bile and urine secretions were detected. Thus, it was demonstrated that the principal site of fumonisin metabolism is in the gut (and not in the liver) [35].

In the case of pigs, they were fed 45 mg FB1/kg b.w., for 10 days. It was established that from 1 to 3.9 % of the administered dose was converted into aminopentol and partially hydrolysed FB1 in chymus (intestine contents). 41%, 47% and 12 % of total FB1 in feces were recovered as unhydrolyzed, partially hydrolysed and totally hydrolysed (AP1) forms, respectively. While, 65%, 24% and 16% of total FB1 in urine was detected as intact, partially hydrolysis and full hydrolysis forms, respectively [74].

In the case of ruminants, Rice and Ross (1994) estimated that rumen microflora could metabolize 60-90% of FB1 oral dose to hydrolyzed forms (aminopolyols and aminopentol) in cattle and sheep feces [36]. In contrast, Smith and Thakur (1996) mentioned that more than 80% of FB1 oral dose were excreted in feces as unmetabolized form, and that there was no detection of hydrolyzed form in feces or urine [52]. These results were confirmed by F. Caloni (2000-2002), which proved that FB1 is poorly metabolized in the rumen, after incubating 1 mg/ml of FB1 in ruminal fluid for up to 72 hours. HFB1 is not detected at the end of experiment [69-136].

There is no evidence concerning FB1 metabolized by liver microsomal enzymes, such as cytochromes P450. Spotti-m (2001) published the results that bovine liver microsomal enzymes are unable to metabolize FB1 to any hydrolyzed forms such as aminopolyol 1, aminopolyol 2 or aminopentol after incubating FB1 with bovine microsomes for 1 hr [61]. This result conforms to previous data obtained in rats, which had established that FB1 was not metabolized by microsomal enzyme [62]. Therefore, the tolerance of ruminants to FB1 is apparently not related to their detoxification activity by rumen or hepatic microsomes activity. However, it is dependent on their lower bioavailability.

Second metabolism of HFB1 to N-acyl-derivatives (Cn-HFB1 = C16, C18, C20, C22, C24 and C24:1) by the ceramide synthase were documented for the first time in rats, after intraperitoneal injection of 1120 µg/kg b.w. for four days. Those acylation metabolites products have fluctuated cytotoxic effects comparing to HFB1. C16 and C24:1 has potential reducing effect on the number of viable HT29 cells, and inhibits ceramide synthase in cell culture [72-192].

There is no data regarding avian ability to hydrolyze FB1.

Concerning FB2, in feces of monkey after oral exposure to single oral dose 7.5 mg FB2/kg b.w., 6% of the dose was recovered as not metabolized, 47% was detected as partially hydrolyzed and 1.1% as fully hydrolyzed (aminopolyols) [51]. By contrast, pig gut microflora was unable to metabolize FB2. This description was carried out after feeding pigs with 8.6 mg FB2/kg b.w. for 10 days. 23% and 6% of total FB2 in feces and urine respectively were detected as intact molecules (unhydrolyzed form) [74].

Consequences of fumonisin metabolism in terms of toxicity are not fully documented. A study was conducted in piglets exposed by gavage to purified 2.8 µmol FB1 or HFB1/kg b.w. /day, for 2 weeks. Histopathology results recorded mild to moderate hepatic damage, which indicated by presence of nuclear vacuolization of hepatocytes, megalocytosis and signs of hepatic necrosis in animal group exposure to FB1. By contrast, animals exposed to HFB1 have similar results as control animals. That means that HFB1 does not induce liver damages. Biochemical analyses also introduced evidence of liver damage by increased concentrations of serum albumin, total protein, cholesterol, triglycerides, fibrinogen and gamma-glutamyl transferase (GGT) in groups treated with FB1 at the 7th and 14th days after exposure. Conversely, no change in the serum biochemical analysis was detected at the 7th and 14th days in animals which had ingested HFB1. Mild to moderate small intestinal lesions indicated by lymphatic vessel dilation and interstitial edema in the proximal small intestine, a decrease in villi height and atrophy were obtained in FB1 treated animals. No significant intestinal lesions were obtained in HFB1 and control groups. Sa:So ratio increased by 8–10 times in the serum and 28 times in the liver of treated animals with FB1 compared to control animals. By contrast, only slight augmentations in Sa:So ratio was recorded in liver treated animals with HFB1. FB1 had significant

alterations in cytokines production in liver and small intestine lymph nodes, which led to increase IL-1 β and IL-8, and decrease IL-2, IL-6, INF α and INF γ in them. Conversely, HFB1 had only slightly decreasing effect on IL-6 of liver and small intestine lymph nodes [166]. Thus, it is suggested that the less toxic effect of HFB1 is related to its slight disrupts of sphingolipids metabolism and slight immunosuppression. Therefore, conversion of FB1 to hydrolyzed forms could be a good strategy to reduce FB1 toxicity. Nevertheless, the secondary metabolism of HFB1 does have cytotoxic effects *in vitro* and small immunosuppression *in vivo* [166].

4. Excretion

Data obtained in different animal species by using FB1 are synthesized in table 4, and compared to results obtained with FB2 in table 5. Studies conducted with radiolabelled or unlabelled FB1 demonstrate a rapid elimination half-life (10 to 180 min) and a rapid clearance (below 30 ml/min/kg). Because FB1 is poorly absorbed, the elimination kinetics of FB1 is not easy to describe after oral administration. In addition, bile secretion plays an important role in elimination of FB1.

In rats, 67% and 25% of administered single dosage of 7.5 mg FB1/kg b.w, by ip injection and gavage were recovered in bile secretion and urine, respectively, after 24hr post toxicant [33-64].

In pigs, a single dose of 0.4 mg [14C] FB1 /kg b.w. was applied intravenously to normal pigs and bile duct cannulated pigs. The results obtained proved that, 70.8% of the applied dose was recovered in the biliary within 72 hours. Furthermore, the elimination half life in cannulated pigs was ten times faster than in non-cannulated pigs (T_{1/2 β} was 17.1 and 182 min, respectively). That means FB1 is accumulated in the bile gland then re-absorbed from the intestines [31].

Excretion of FB1 seems more important in ducks than in turkeys and laying hens. Clearance in ducks, turkeys and laying hens were 19.6, 7.6 and 1.18 ml/min/kg, respectively. Elimination half-life was 26, 85 and 48 min, respectively, (table 4) [23-24-30].

Table 4: Excretion of FB1

Animals	Route	Dose ¹	Parameters	Ref
Rats	Oral	0.69*	0.5% of dose recovered in urine, 1.4% excreted in bile at 4 hr after dosing	[71]
	IV	2	T1/2 β : 62 min CL: 1.2	[32]
	IP	7,5	16-25% recovered in urine 48 hr after dosing 67% recovered in bile 24 hr after dosing	[33-64]
	Oral	7,5	2% recovered in bile 24 hr after dosing	[64]
	Oral	10	T1/2 β : 205 min	[32]
Monkeys	Oral	6,4*	62% of dose recovered in feces 24 hr after dosing	[35]
	IV	1,6	T1/2 β : 40 min	[55]
Pigs	IV	0.4*	Pig: T1/2 β :182 min Cannulated pig : T1/2 β :17.1 min 70.8% of dose recovered in bile 72 hr after dosing	[31]
	Oral	0.5*	90% of dose recovered in feces , less than 1% excreted in urine and bile 72 hr after dosing	
Cow	IV	0,05-0,2	T1/2 β : 17 min	[34]
Laying Hens	IV	2*	T1/2 β : 48.8 \pm 11.2 CL: 1.18 \pm 0.15 98.6 % of dose recovered in excreta 24 hr after dosing	[30]
	Oral	2*	T1/2 β : 116. 6.6 % of dose recovered in excreta 24 hr after dosing	
Turkeys	IV	10	T1/2 β : 85 min CL: 7.6	[24]
	Oral	100	T1/2 β : 214 min CL: 7.5	
Ducks	IV	10	T1/2 β : 26 min CL: 19	[23]
	Oral	100	T1/2 β : 70 min CL: 17	

¹ single dose (mg/kg b.w.) of FB1 or *[14C] FB1;

T1/2 β (min): Elimination half life; Cl (ml/min/kg): Clearance

Concerning FB2, in rats, eliminated quantity via renal route is lower (1.2% of dose) than FB1 (16-25% of dose) by using same dose of toxin and protocol of experimental (table 4 and 5) [28-64]. Also in pigs, the eliminated quantity of FB2 was lower than FB1 by 9 and 14 times through urine and feces, respectively, after oral administration of 50, 20, and 5 mg of FB1, FB2, and FB3 respectively, per animal, per

day, for 22 days [206]. In contrast, in monkeys the elimination half-life of FB1 (40 min) was longer than the one of FB2 (18 min) (table 4 and 5) [51-55].

Table 5: Excretion of FB2

Animals	Route	Dose ¹	Parameters	Ref
Rat	IP	7.5	T1/2 β : 26 min. 84.1% recovered in feces 72 hr after dosing 1.2 % recovered in urine 72 hr after dosing	[28]
	Oral	7.5	82% recovered in feces 72 hr after dosing 0.2 %recovered in urine 72 hr after dosing	
Monkeys	IV	2	T1/2 β : 18 min Less than 50% recovered in feces 168 hr after dosing 4.1 % recovered in urine 168 hr after dosing	[51]
	Oral	7.5	Less than 50% recovered in feces 168 hr after dosing 0.2 % recovered in urine 168 hr after dosing	

¹single dose (mg/kg b.w.) of FB2;

T1/2 β (min): Elimination half life; CL (ml/min/kg): Clearance

5. Residual

Residual of FB1 labelled and unlabelled in tissues are presented in tables 6 and 7. The accumulation of FB1 was more significant in the kidneys than in the liver in rats treated with single oral dose of 10 mg FB1/kg b.w. [32]. These results are supported by other data obtained with rats, which demonstrates that FB1 residual in kidneys was 10 times higher than in the liver [26]. By contrast, residual of FB1 was more pronounced in liver than in kidney in pigs feed 45 mg/kg b.w., for 10 days [26], in monkey exposure to single oral dose of 6.42 mg¹⁴C-FB1/kg b.w. [55], in turkeys feed 20 mg FB1+FB2/kg b.w., for 9 week [24], and in ducks feed 20 mg FB1+FB2/kg b.w., for 12 days [23], (table 6 and 7).

Table 6: Residual of [14C-FB1]

Animals	Route	Dose ¹	Organs residual	Ref
Monkeys	IV	1.72	Dose recovered 24 hr after dosing: Liver: 1.92%. Kidney: 0.37%. Muscles: 0.62%. Brain, Lung, heart and spleen: traces	[35]
	oral	6.42	Dose recovered 24 hr after dosing: Liver: 0.64%. Kidney: 0.03%. Muscles: 0.14%. Brain, Lung, heart and spleen: traces	
	oral	8	24 hr post dosing. Muscle: 1%. Liver: 0, 4%. brain : 0,2%	[55]
Pigs	IV	0.4	1076 ng/g recovered in liver 72 hr after dosing. 486 ng/g recovered in kidneys 72 hr after dosing.	[31]
	Oral	0.5	107 ng/g recovered in liver 72 hr after dosing. 48 ng/g recovered in kidneys 72 hr after dosing	
Laying hens	IV	2	Traces recovered in liver, kidney and crop, but not in eggs 24 hr after dosing	[30]
	Oral	2	Traces recovered in liver, kidney and crop, but not in eggs 24 hr after dosing	

¹single dose (mg/kg b.w.)

In another experiment, pigs were exposed to contaminated feed with mixture of FB1, FB2 and FB3 (45, 8.6 and 4.6 mg/kg b.w., respectively) for 10 days. One should ignore the numbers obtained in this study, because the sampling time was not recorded precisely. Nevertheless, the results showed that, accumulation of FB1, HFB1, AP1 and FB2 could be detected in many parts of the carcass such as liver, kidneys, lung, spleen, brain, muscle and fats at different percentages. Liver has the highest residual quantity of FB1 compared to other organs, and equal residual quantity was obtained in kidneys and muscles. HFB1 residue is more concentrated in muscles compared to other tissues. HFB1 is not recovered in the brain. AP1 residual is more pronounced in muscles and fats compared to other tissues. The percentage shares of FB1 and its metabolic forms residue in the overall body tissues are: 50% in the form of intact FB1, 20% in the form of partially hydrolyzed and 30% in the form of aminopentol. Ten days after the last toxin exposure dosages, FB1 residual could be

detected in all organs and tissues tested. The highest one was observed in the liver, and smallest one detected in the brain. Only small quantity of HFB1 remained in the spleen and fat, while AP1 still presents overall small quantities in all animal tissues. Concurrently, ten days after the last administration dose of FB2, small quantities were recovered in muscles and kidneys, (table 7) [74].

Table 7: Residual of fumonisins after oral exposure

Animals	Dose	Organs residual (µg/kg or µg/L)	Ref
Rats	One dose 10 mg FB1/kg b.w.	FB1: 2% in liver, and 30% in kidneys	[32]
Pig	10 days feeding FB1, FB2 and FB3 45, 8.6 and 4.6 mg/kg feed, respectively	FB1: 17 liver, 10 kidney, 8 muscles and < 5 in other tissues. HFB1: 5 muscles, < 2.5 in other tissues AP1: 7.5 kidneys, 5.6 abdominal fat, < 1 in other tissues. FB2: 6 muscles, < 1 in other tissues	[74]
	3 week 0.91 mg FB1+FB2/kg feed then 4 weeks: 2.34 FB1+FB2/kg feed	FB1: 28 liver FB2: < 10 in all tissues	[73]
Cattle	One dose 1-5 mg FB1/kg b.w.	FB1 and AP1: < 0.007 and 0.025 in milk, respectively	[34-38]
	3 mg FB1/kg b.w. / day, 14 days	FB1 and AP1: < 0.005 in milk	[137]
Turkey	20mg FB1+FB2/kg feed, 9 week	8 hours after the last meal: Liver: 117; Kidney: 22 Muscles: < 13	[24]
Duck	20 mg FB1+FB2/kg feed, for 12 days	8 hours after the last meal: Liver: 20; Kidney and muscles: < LD 13	[23]

At present, there is no information regarding ability of FB1 to persist in cattle tissues or bovine products. For example, cows were inculcated directly into the rumen with 1.0 mg or 5.0 mg FB1/kg of b.w. The results showed that there was no FB1 detected in the serum or milk. This phenomenon was expected due to lower bioavailability of FB1 in ruminants, (table 7) [34].

In avian species, such as laying hens, it has been demonstrated that less than 1% of oral dose [14C] FB1 is residual in the tissue, but none in the eggs, (table 6) [30].

Similar results have been obtained with unlabelled FB1 in ducks and turkeys after feeding them for several days with contaminated maize with 20 mg FB1 + FB2/kg of feed. The findings show that less than 1% of the oral dose is residual in the livers of ducks and turkeys. In addition, FB1 residual in liver is more distinct than the one in kidneys and muscles in both species. Also, the FB1 residual is more pronounced in turkey livers than duck livers (117- 20µg/kg of tissue, respectively) (table 7) [23-24]. Residual of FB1 in kidneys and liver are less pronounced in ducks and turkeys than in rats even if the animals have similar bioavailability. This result is probably due to the high clearance in ducks and turkeys, compared to rats (19.6, 7.5 and 1.2 min, respectively) (table 4) [23-24-32].

Data concerning the residual of FB2 has been poorly documented. However, residual of FB1 and FB2 was found in the lungs, heart, liver, kidneys, spleen, brain, serum, bile, muscle and fat by varying percentages in pigs fed on a mixture of FB1, FB2 and FB3 (50, 20 and 5 mg /head/day, respectively), during a 22 day period. The highest FB1 concentrations were found in the liver and kidneys (99.4 and 30.6 ng/g of tissue, respectively), While the highest concentrations of FB2 were detected in the fat and liver (2.6 and 1.4 ng /g of tissue, respectively). The ratio of FB1/FB2 residuals in different pig organs is 19/1%, whereas in fat samples it is 4/1%. That means residual of FB1 is more important than FB2 in all body tissues [206]. That revealed to residual of FB1 was more pronounced than FB2 in all body tissues.

Other study has recovered the FB2 residual in all body tissues of pigs, with high concentration in fat, muscles tissues, and liver after ten days of last administered dose, (table 7) [73-74]. These data allude to the risk of FBs residual being present if introduced to a consumer (animals or human) with other mycotoxins.

6. Conclusion

The literature reviews reveal that fumonisins generally have low bioavailability (less than 5% of an administration dose). However, differences between species can be observed. For example, absorption is four times higher in pigs than in cattle, and residual of fumonisin is detected in all parts of a pig carcass, while it is not recovered in cow's milk or tissues. Concerning the avian species, bioavailability of FB1 it is around three times lower in layer hens than in turkeys. In ducks and turkeys, it appears that the bioavailability of FB1 in turkeys represents 160% of the value in ducks (table 8).

Table 8: Toxicokinetic and residual of FB1 in ducks and turkeys

Parameter	Duck	Turkey
FB1 single dose (100 mg/kg b.w.)		
T max (min)	120	180
C max (µg/ml)	0.628 ± 253	0.991 ± 0.061
T1/2 α (min)	74 ± 4	29.4 ± 3.3
T1/2 β (min)	71 ± 3	214 ± 36
T1/2 Ka (min)	66 ± 4	44 ± 4
AUC (µg/ml/min)	121 ± 9	443 ± 32
F (%)	2 ± 0.1	3.2 ± 0.2
MRT (min)	200 ± 12	408 ± 43
MAT (min)	176	356
Cl (ml/min/kg)	16.7	7.5
Vdarea (L/kg)	1715 ± 82	2313 ± 388
Vc (ml/kg)	179 ± 14	111 ± 21
FB1+ FB2 (20 mg /kg feed)	2 weeks	9 weeks
FB1 liver (µg/kg)	20± 6	117 ± 50
FB1 kidneys (µg/kg)	<LD	22 ± 8

Values are expressed as mean ± SE

Tmax: time of occurrence of maxima concentration of FB1 in serum; Cmax: maxima concentration of FB1 in serum; T 1/2 α : half-life at α ; T 1/2 β : terminal elimination half-life; T1/2Ka: absorption half-life; AUC: area under plasma concentration-time curve from t = 0 to infinity; F: extent of systemic absorption based on the determination of the ratio between AUC obtained after oral administration and the AUC obtained following the oral administration corrected by the dose used; MRT: mean residence time; MAT: mean absorption time; Cl: total plasma clearance; Vdarea: volume of distribution; Vc: volume of the central compartment; LD: limit of detection = 13µg/kg

Interspecies variations are also observed concerning the elimination half-life and clearance. Although elimination half-life is short in all tested animals species ($T_{1/2\beta}$: 10 to 180 min), the toxicity of FBs appeared cumulative (see below) and residual appeared to cumulate in pigs. Concerning the avian species, clearance of FB1 it is around six times lower in layer hens than in turkeys. In ducks and turkeys, it appears that the clearance of FB1 in ducks represents 220% of the value in turkeys (table 8). Finally, together absorption and clearance could explain that the residual of fumonisins in turkey livers are 585% of those in ducks liver (table 8).

Only few data are available concerning the metabolism of fumonisins and the toxicokinetics of FB2 other than FB1. There is a disagreement about the possibility of metabolism FB1 to HFB1 in ruminants whereas HFB1 and AP1 were detected in pig carcass. No data is available in the avian species. Concerning FB2, absorption and elimination seem lower than FB1 in rats and monkeys whereas, residual of FB2 is recovered in all body tissues of pigs, suggesting that the toxicokinetic of FBs is quite different from FB1. No data is available in the avian species.

III. Fumonisin toxicity

This chapter will present the most important toxicological studies in laboratory and farm animals which are at the origin of the recommendations of maximum levels for fumonisins in animals and human foods.

Toxicity in laboratory animals was presented according to the duration of the study (acute, short term, long term exposure) to present organ toxicity and risk of cumulative effect. Genotoxicity and reproductive toxicity were also presented as target for fumonisins toxicity. When data are available, specific analysis of the effect of sex and strain were reported. A table was done to present the NOEL (no observed effect level) in this species.

Toxicity in farm animal was presented depending on the species naturally exposed to fumonisins to show species difference in toxicity (dose) and target organs. Data concerning the avian species were specially analyzed and synthetized to understand interspecies variation in this group of animal species, that is often considered as a homogeneous group. High, low and intermediate sensitivity species were separated depending on the dose necessary to produce an effect. A species was considered as "resistant" when no adverse effect is observed in farming condition (no report of toxicity) whereas it was called "sensitive" when toxicity (clinical signs or mortality) could occur. At the end of this chapter, two tables report the European and FDA (food drug administration) guidance levels for fumonisins in animal feed. A specific table was done for the FDA-recommended maximum levels for fumonisins in human food.

Specific effects of fumonisins on sphingolipids metabolism and biochemistry are shortly presented in this chapter. A specific analysis of the consequences of FBs exposure on theses parameters is done in paragraph VI, after presentation of mechanism of action of the toxins.

1. Laboratory animals

1.1. Acute toxicity (single dose exposure)

No studies have been published on the lethality of single doses of pure FB1 on laboratory animals. In previous data, it was demonstrated that FB1 had no fatal effect after mice had been given a single dose of 25 mg/kg b.w. by gavage or subcutaneous injection. Results showed reversible alterations in cytokine expression, serum enzymes activity, and blood cell counts [81].

Renal tubules proliferation, death of cells (apoptosis) and severe nephrosis were observed in male Sprague-Dawley rat after intravenous single dose of 1.25 mg FB1/kg b.w. Cell proliferation was also detected in the liver [75].

Male Wistar rats were treated with single FB1 doses 5, 50, and 500 µg/kg b.w. by gavage route. The animals were sacrificed at the 4th, 24th and 48th hours after treatment. No difference between control and treated animals was found in relation to oxidative stress. Histopathological changes in liver were spelled out by a significant increase in apoptotic cells and cell necrosis. Cell necrosis was observed at the end of study with all dose levels. Increase in apoptotic cells was observed 24 hours after applying a dose of 5 µg/kg b.w. meanwhile, at doses of 50, and 500 µg/kg b.w. it took between 4 and 48 hours [86]. This study proved that fumonisin toxicity is time and dose-dependent

1.2. Short-term studies of toxicity

1.2.1. Animal Species

Previous studies had reported that rabbits were more sensitive to FB1 nephrotoxicity effects than rats and mice. These findings were recorded after exposing animals to different doses of toxins, via different administration routes as follows:

Rabbits were treated intravenously with 0.15, 0.3, 0.5, and 1.0 mg FB1/kg b.w., for 4 or 5 days. After multiple doses, signs of animal toxicity appeared in lethargy, and decreased urine production. At the end of the experiment, signs of nephrotoxicity, such as elevation of serum creatinine and urea nitrogen, and urine

protein, and signs of hepatotoxicity like elevation in liver biochemical parameters (enzymes and total bilirubin), ballooning degeneration, hepatocellular swelling, and bile stasis were observed with all doses. Disturbance of sphingolipids metabolism appeared in the liver, kidneys, muscles, serum, and urine, but not in the brain. This disturbance of sphingolipids metabolism was more pronounced in the kidneys than in other tissues [78].

Males and females of B6C3F1 mice and Fischer 344 rats were fed diets containing 0, 1, 3, 9, 27, or 81mg FB1/kg/day for 13 weeks. In both species, no differences were recorded between control and treated groups concerning animal behaviour, appearance, body weight, or food consumption. Male and female F344 rats show nephrotoxicity by consuming 27 mg FB1/kg/day. No effect on nephrotoxicity was obtained in both sexes of mice for all doses. These results demonstrated that Fischer 344 rats were more sensitive to renal toxicity than B6C3F1 mice [79].

Male and female B6C3F1 mice were fed diets containing 100-500 mg FB1/kg for 28 days, whereas, Fischer 344 rats were fed diets containing 99, 163, 234 or 484mg FB1/kg for 28 days. No signs of nephrotoxicity were obtained in mice, whereas, hepatotoxicity was obtained in males and females mice fed with diets containing 250-500 mgFB1/kg. In both sexes of rats, nephrotoxicity was achieved with the lower contaminated diet; hepatotoxicity signs such as biliary hyperplasia and hepatocellular degeneration were observed at the 163 mgFB1/kg level in diet. These results matched with previous studies which had reported that rats were more sensitive to nephrotoxicity than mice [80].

1.2.2. Animal Strains

In the case of male BD IX rats which consumed a diet containing 1 g FB1/kg of feed for 28 days the mean body weight reduction was 50% lower than the control group [8]. Concurrently, in male Fischer 344 rats fed 1g FB1/kg of feed for 26 days the mean body weight reduction was 80% lower than the control group [89].

In male Sprague-Dawley rats and male F-344 rats, nephrotoxicity symptoms such as nephrosis, necrosis epithelial cells, and apoptosis were observed at all exposure doses of FB1. By contrast, no signs of nephrotoxicity were observed in

BDIX- male rats after consuming high doses of FB1. These results came to light after feeding male Sprague-Dawley rats with contaminated diets at concentration of 0, 1.4, 4.4, and 13.5 mg FB1/kg b.w. per day for 4 weeks, male F-344 rats were fed on diets containing 0.7, 3.5, 6.8, 15 and 25 mg FB1/kg b.w. per day for 21 days, and BDIX-male rats fed diet contain 70mg FB1/kg b.w. per day for 90 days [76-79-81-84].

Male transgenic p53+/- and corresponding wild-type mice were fed diets containing FB1 (97%) at levels of 0, 5, 50 or 150 mg/kg diet, for 26 weeks. In both strains liver weight was not affected. In mice transgenic p53, hepatic necrosis and apoptosis were observed at medium and high dose treated groups, whereas in wild-type mice, hepatic necrosis and hepatic apoptosis was only recorded in the high dose treated group [88].

It can be summarized that Sprague-Dawley and Fischer rats are more sensitive to body weight reduction and nephrotoxicity caused by FB1 toxicity than male BDIX-rats. Also, wild-type mice are more resistant than p53 mice to the FB1 effects of decreased body weight and hepatic apoptosis. Based on the above mentioned information, it can summarize that fumonisin toxicity is different between same animal species, and animal strains play important role in the progression of FB1 toxicity

1.2.3. Animal Sex

Male and female Sprague-Dawley rats were fed diets containing 0, 15, 50 and 150 mg FB1/kg, for 4 weeks. Daily intake was estimated to be 1.4, 4.4, and 13.5 mg/kg b.w. In both sexes, hepatotoxicity signs such as modification of biochemistry and hepatocellular necrosis were observed only with dietary level 150 mg FB1/kg. The NOEL of liver was 4.1-13 mg/kg b.w. per day. On the other side, nephrosis was obtained in males fed contaminated diet of more than 15 mgFB1/kg, and in females fed contaminated diet of more than 50 mg/kg. The NOEL of kidneys is less than 1.4 mg/kg b.w. per day in males, and about 1.4 mg/kg b.w. per day in females. These results demonstrated that male Sprague-Dawley rats are more sensitive to FB1 nephrotoxicity than female [76-79].

Male and female F344 rats consumed diets contaminated by 99, 163, 234 and 484 mg FB1/kg of feed, for 28 days. Hepatic toxicity signs (biliary hyperplasia,

hepatocellular degeneration, and hepatocellular apoptosis) were more pronounced in female rats which had received contaminated diet of more than 163 mg FB1 /kg, and in male rats which had received contaminated diet of more than 234 mg FB1 /kg. By contrast, renal toxicity signs more was pronounced in males fed with dose of 99 mg FB1/kg, and in females fed with dose of 163 mg FB1/kg. These results proved that female rats are more sensitive than males regarding hepatotoxicity. By contrast, renal toxicity is more pronounced in male than in female [77-80].

B6C3F1 mice and Fischer 344 rats were fed diets containing 0, 1, 3, 9, 27, or 81 mg FB1/kg/day for 13 weeks. No difference in behavior, appearance, and body weight or food consumption between control and treated groups was detected.

Male rats were more sensitive than female rats F344 to nephrotoxicity ($3 < \text{male NOEL} \leq 9 \text{ mg FB1/kg/day}$, and $27 < \text{female NOEL} \leq 80 \text{ mg FB1/kg/day}$). Hepatotoxicity was observed in female rats fed contaminated diet of more than 27mg of FB1 ($27 < \text{female NOEL} \leq 80 \text{ mg FB1/kg/day}$), whereas, male rats were not affected even with contaminated diets of 81 mg FB1/kg for 90 days ($81\text{mg} < \text{male NOEL}$).

Concerning B6C3F1 mice, nephrotoxicity was not recorded in male and female, whereas, hepatotoxicity was only observed in female. Those results illustrated that male Fischer 344 rats were considerably more sensitive to renal toxicity than female rats. By contrast, female Fischer 344 rats were more susceptible than male rats to liver toxicity. Also female B6C3F1 mice were more sensitive to hepatotoxicity than male mice [79].

1.2.4. Animal Organs

Male and female Sprague-Dawley rats were fed diets containing 0, 15, 50 and 150 mg FB1/kg, for 4 weeks. Estimated daily intake was about 1.4, 4.4, and 13.5 mg FB1/kg b.w. per day. Data compiled for both sexes demonstrated that liver was less sensitive to fumonisin toxicity than kidneys. The average of liver NOEL was about 4.1-13 mg/kg b.w. per day, while kidney NOEL was equal or less than 1.4 mg/kg b.w. per day, (table 9). [76-79].

The results obtained with Sprague-Dawley rats were supported by a study conducted on rats F-344 fed with contaminated diets 10, 50,100,250 and 500 mg

FB1/kg for 21 days. Intake was estimated at 0.7, 3.5, 6.8, 15 and 25 mg FB1/kg b.w. per day. Nephrosis, cells necrosis and apoptosis were obtained with a lower contamination diet 10 mg FB1/kg, or 0.7 mg FB1/kg b.w. per day. Meanwhile, hepatic cell necrosis, apoptosis, and endothelial cell proliferation were observed on animals fed a diet of 50 mg/kg or 3.5mg/kg b.w. per day. These results proved that kidneys were more sensitive to fumonisin toxicity than liver [84].

Male BALB/c mice received a subcutaneous dose of FB1 at 0.3, 0.8, 2.3, or 6.8 mg/kg b.w. per day for 5 days. Decreased kidney weight was observed one day after the last injection at all doses. While liver weight did not show any effects by all dosages. Dose-dependent increase apoptosis and accumulation of free sphingolipids were obtained in liver and kidneys. Apoptosis was detected in the livers of mice at doses > 0.8 mg/kg b.w. per day and in the kidneys at all doses. If it is assumed that 10% of an oral dose would be absorbed in mice, the estimate of NOEL for oral administration would be less than 0.3 and 0.8 mg FB1/kg b.w. per day in kidney and liver, respectively, (table 9) [82-83].

1.3. Long-term studies of toxicity and carcinogenicity

F344 rats and B6C3F1 mice were fed for two years a diet containing the following concentrations of FB1: female rats, 0, 5, 15, 50, and 100 mg FB1/kg of feed; male rats, 0, 5, 15, 50, and 150 mg FB1/kg of feed; female mice 0, 5, 15, 50, and 80 mg FB/kg of feed; male mice, 0, 5, 15, 80, and 150 mg/kg of feed. Decrease in body weight was observed only in female F344 rats which had consumed contaminated diet of 100 mg/kg. Whereas, no difference in body weight between control and treated groups was observed in Male F344 rat, female and male mice fed highest level of contaminated diets. Tubule adenomas and carcinomas were demonstrated in male F344 rats with mild and high dose 50-150 mg FB1/kg. No tumorigenic signs were mentioned in females with a high dose of 100 mg FB1/kg. Hepatocellular adenoma and carcinoma were demonstrated in female mice with all concentrated diets. Whereas, those signs were not observed in male mice fed 150 mg FB1/kg. This study proved that FB1 is a rodent carcinogen that induces renal tubule tumours in male F344 rats and hepatic tumours in female B6C3F1 mice [90].

Male BDIX rats received diet containing 50 mg FB1/kg of diet, equivalent to 1.6 mg FB1/kg b.w. per day, for 26 month. Signs of hepatic preneoplastic changes such as hepatic nodules and cirrhosis were observed at 18 months after exposure. Development of primary hepatocellular carcinoma was reported 18-26 months after exposure. At the end of the study, no lesions were demonstrated in the esophagus, and heart [10].

The dose-response relationship between FB1 and hepatocarcinogenesis was investigated in BD IX rats fed a diet containing FB1 at a concentration of 1, 10, or 25 mg/kg of feed, for 2 years. The carcinogenicity markers such as apoptosis, proliferation of duct epithelial cells, and mild fibrosis led to a slight distortion of the liver architecture in some rats. Necrosis, apoptosis and calcification were observed in the tubular epithelium cells of the kidneys. All these lesions were mainly present with 25 mg/kg of diet and to a lesser extent with 10 mg/kg of diet, or mean daily intakes of 0.8 and 0.3 mg/kg b.w, respectively [45].

Male BDIX rats were fed maize contaminated with *F. verticillioides* MRC 826 culture material for 849 days. This strain was isolated from an esophageal cancer outbreak area in South Africa. Rats were fed a diet containing 6.9 mg FB1/kg b.w. per day, for 288 days. Then, they were administered a diet containing 3.2 mg FB1/kg b.w. per day, for 606 days. 80% and 63% of the cases developed hepatocellular carcinoma and ductular carcinoma in the liver, respectively. Indeed, in several cases there were occurrences of cirrhosis and nodular hyperplasia in the liver, pulmonary metastases, adenofibrosis, neoplastic lesion and endothelial hyperplasia of the endocardium membrane. Esophageal hyperplasia was obtained in 50% of the treated rats. No lesions were found in the kidneys [19].

Male and female vervet monkeys were fed contaminated diets with *F. verticillioides* MRC 826 for 13.5 years. The equivalent to average doses was approximately 8.2-13 mg FB1/kg diet. Toxicity monitors such as clinical chemical analysis, serum biomarkers and blood accounts were conducted bimonthly. Liver biopsy samples were taken at regular intervals for the first 4.5 years. Typical liver lesions were obtained at high doses including: portal fibrosis, hepatocytes nodules, bile duct proliferation and apoptosis. Kidney histopathological changes examinations were not conducted. Lower observed effected level (LOEL) for sphingolipids changes

in serum was 22 to 48 mg/kg diet, equivalent to 0.29-0.64 mg/kg b.w. per day. Other parameters that were also affected throughout the study included lipid parameters associated with hypercholesterolemia. Blood account referred to significant decreased in white and red blood cell and platelet counts [81].

1.4. Genotoxicity

Genotoxicity of FB1 has been measured in *vivo* and in *vitro*. The results of several independent studies showed evidence indicating that FB1 can damage DNA indirectly by increasing oxidative stress. Oxidative damage was closely associated with FB1-induced hepatotoxicity and induction of preneoplastic lesions in *vivo*. Serum microsomal membranes, mitochondria and nuclei appeared to be significantly affected by lipid peroxidation [81]. Kidneys DNA damage due to increased ROS production was observed in male Wistar rats exposed to intraperitoneal injections of 500 µg FB1/kg b.w. per day for 7 days [93]. Also, liver DNA damage was obtained in Wistar rats after single oral doses of 5, 50 and 500 µg FB1 /kg b.w. [86]. Male F344 rats consumed initiated phase diets consisting of a control diet or a diet containing FB1 at 250 mg/kg for 3 weeks. Those were followed by promotion phase diets consisting of control diets or diets containing phenobarbital at 500 mg/kg for up to 30 weeks. The results obtained showed that liver foci associated with hepatotoxicity were observed only in rats treated with FB1 and followed by Phenobarbital.

Consequently, those results suggested that FB1 may have cancer-promoting potential via oxidative damage and genotoxicity properties [94].

1.5. Reproductive toxicity

The neural tube is responsible for forming the brain and spinal cord. Failure of the neural tube to close in the first few weeks of embryonic development leads to congenital malformations called neural tube defects (NTDs). The real etiology of NTDs is unknown, but there are many implicated factors such as: FB1 contaminated diet, B12 deficiency or over activation of S1P receptor-mediated signaling pathways [81-89-208].

In 1990-1991, NTDs were highly occurring in the state of Texas, United States of America, affecting approximately 29 of every 10,000 babies born. Concurrently, the

Health department of Texas suggested that Texan population was consuming large quantities of corn. Therefore pregnant women may have been exposed to high levels of FB1, which increased the risk of NTDs [209-210]. The statistical data of the Health department of Texas from the period 1999 - 2004 recorded significant decline in NTDs down to approximately 5 to 6 of every 10,000 babies in pregnant women who took supplements with folic acid (B12) [211].

In vitro the inhibitor effect of FB1 on biosynthesis of the folate receptor (GPI-anchored protein) is proved. This inhibition leads to deficiency in folate (B12), which is associated with an increased risk of neural tube defects (NTDs). However, the inhibition of the folate transporters by FB1 in vivo has not been confirmed by feeding studies [81-89].

Neural tube defects (NTDs) were induced in pregnant LM/BC mice at embryonic days 7.5 and 8.5, via intraperitoneal injection with pure FB1 0, 5, 10, 15, 20 mg/kg b.w. per day, and the fetuses were collected at embryonic day 17.5. The results proved that embryonic malformations were dose-dependent. 79% of the mice fetuses exposed to the highest dose of 20 mg/kg b.w. per day had exencephaly (when brain is located outside of the skull), whereas NTDs was observed at all dosage levels. Also, FB1 induced a significant alteration of sphingolipids metabolism in the liver, kidneys, and placenta of pregnant dams mice, as well as in the embryonic tissue. Therefore, suggesting that FB1 is capable of crossing dam placenta and inhibiting de novo sphingolipids biosynthesis within the embryo [95].

Over activation of S1P receptor by excessive production of sphingoid bases 1-phosphate (S1P) is increase the risk of NTD as mentioned in LM/Bc and SWV mice, after they were injected by IP with 20 mg/kg b.w. at embryonic days 7.5 and 8.5 [208].

Pregnant New Zealand White rabbits were exposed to purified FB1 at 0.10, 0.50, or 1.00 mg/kg b.w. /day, via gavages, on gestations days 3 to 19. Maternal body weight was not affected. Male and female pups which were exposed to 0.50 and 1.00 mg/kg/day had a reduction in their body weight when compared to a control group, by 13 and 16%, respectively. Fetal liver and kidney weights also decreased at these doses. At day 20 of gestation, modification of the sphingolipids metabolism appeared in maternal urine, serum, and kidneys when compared to controls, whereas the

embryo was not affected. Therefore, that suggested that FB1 was unable to cross rabbit placenta. Furthermore, a decrease in pups body and organ weight was a consequence of maternal toxicity, rather than any developmental of fetal toxicity produced by FB1 [212].

1.6. Conclusion

The lethal single dose of FB1 in laboratory animals has not been recorded, nor lethal effect of FB1 administered at a single dose of 25 mg/kg b.w. by gavage or subcutaneous injection in rodents. Possibility of FB1 to crossing dams placenta and inducing embryonic disorders are proven in rats, but not in rabbits.

Liver and kidneys are the major target organs to fumonisin toxicity, which is characterized by apoptotic necrosis and regeneration. Fumonisin B1 toxicity varies depending on:

- **Species:** rabbits are more sensitive to FB1 nephrotoxicity effects than rats and mice.
- **Organs:** in rodents, liver and kidneys are target organs, although differences depending on the species, strain and sex are observed.
- **Strains:** Sprague-Dawley and Fischer rats are more sensitive than BDIX-rats to fumonisin toxicity. Also, wild-type mice are more resistant than p53 mice to hepatic apoptosis.
- **Sex:** males Sprague-Dawley and F344 rats are more sensitive to FB1 nephrotoxicity than females. Whereas, female rats F344 are more sensitive than males to hepatotoxicity. Also, Female B6C3F1 mice are more sensitive to FB1 hepatotoxicity than male ones.

Carcinogenic effects of FB1 such as renal tubule tumours, hepatocellular carcinoma, liver cirrhosis and hyperplasia are reported in rodents consumed FB1 for long time. In addition, genotoxicity effect, by oxidative damage, is observed in rodents. Thus, FB1 is considered as a cancer initiator and a strong cancer promoter for rodents. It is classed as “possibly carcinogenic to humans (Group 2B)”.

NOEL of FB1 in rodents based on hepatic and kidney toxicity are presented in table 9.

Table 9: No observed effect level (NOEL) of FB1 in rodents

Species	Duration	Target Organ	NOEL (mg FB1/kg b.w.)	Ref
Rats and Mice	Short-term	Liver	< 0.75	[50-79-82-83]
	Sub-chronic	Kidney	0.2	
Mice	Sub-chronic	Liver	1.8	[79-207]
	Chronic	Liver	0.6	
Rats	Chronic	Liver	1.25	[150-207]
	Chronic	Kidney	0.25	

It appeared from this table that kidney is more sensitive than liver, mice are more sensitive than rat for chronic toxicity, whereas short-term and sub-chronic toxicity for the liver seems more important than chronic toxicity in rats. The NOEL of 0.2 mg/kg b.w. on kidney toxicity in rat being the lowest observed in all the studies conducted in rodents. Thence, by using a factor of security of 100, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has recommended provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg b.w., for fumonisins B1, B2 and B3, alone or in combination, (JECFA, 2001)[25-267-289].

2. Farm animals: mammals

2.1. High sensitivity species

2.1.1. Equines

Equine species (horses, mules, donkeys, ponies) are apparently the most sensitive to FB1 toxicity. Animal disorders can appear after ingestion of contaminated feed at concentrations >10 mg FB1/kg (equivalent to 0.2 mgFB1/kg b.w. per day), for few weeks [106-107-110]. The target organs in horses are the central nervous system, the liver and the heart [168]. Leukoencephalomalacia, hepatotoxicosis, and cardiotoxicity are dose-dependent.

2.1.1.1. Equine leukoencephalomalacia (ELEM)

ELEM was described for the first time in the United States in 1850, and later on in South Africa [103-104]. Since then, cases have been observed everywhere in the world including the South-west of France [105]. In August 2007 an outbreak of ELEM occurred in Argentina in Arabian horses fed native grasses supplemented with corn kernels and wheat bran. The morbidity and mortality rates were 11.6% and 10%, respectively [143]. It has been hypothesized that equine leukoencephalomalacia is a result of cerebral oedema due to an inability to shut down blood flow to the brain when the horse lowers its head to eat or drink [81]. Equine leukoencephalomalacia syndrome is a sporadic disease characterized by the presence of liquefaction necrotic and yellow discoloration in the cerebral hemispheres, brainstem and cerebellum [144]. The disease appears to be exclusive to equines, but the brain lesions have also been reported in rabbits [101] and pigs [102].

The routes of administration and dose concentration are significant factors in the appearance of the ELEM. One study carried out in 1990 by Kellerman, showed that oral administration of between 1 and 4 mg FB1/kg b.w., over 29 days was able to produce the ELEM in horses [9]. In the same manner, the administration of 0.125 mg FB1/kg b.w., over 7 days by intravenous route is sufficient to cause an onset of ELEM [106]. ELEM has been mentioned in experiments carried out by Wilson and Collar on ponies, which received naturally contaminated corn with 22 mg FB1/Kg b.w., for 55 days [109]. By contrast, another study conducted on horses fed 15 mg FB1/kg feed, for 150 days (equivalent to 0.3 mg of FB1/kg b.w. /day) did not obtain any clinical signs or any alteration in serum biochemical parameters (including disruption of sphingolipids metabolism) [81].

All these results have been supported by analyzing feed coming from confirmed cases infected with ELEM in the USA. The above indicated that consumption of contaminated feed with FB1 at concentration > 10 mg/kg of diet (equivalent to 0.2 mg/kg b.w. per day) was associated with an increased risk for development of ELEM, whereas a concentration < 6 mg/kg of diet (equivalent to 0.12 mg/kg b.w. per day) did not induce ELEM [110]. Therefore, Shephard and Collar recommend the tolerable maximum content of FB1 in feed to avoid risks of ELEM to

be 5 mg FB1/kg of diet [108]. Those results were supported by other investigations in equines, which showed that the minimum oral dose sufficient to induce ELEM appeared to be ≥ 15 mg/kg of diet, and the minimum oral dose of pure FB1 that induces equine leukoencephalomalacia is unknown [81]. On the other hand, cerebral lesions were obtained by intravenous injection of pure FB1 at concentration from 0.01 to 0.05 mg FB1/kg b.w. /day. If considered that an oral dose represents 5% of an intravenous dose, the equivalent of oral dose inducing a brain lesion will be 0.2–1.0 mgFB1/kg b.w. per day.

More recent data obtained in 2007 in Argentina supported all previous studies. It reported that horses which consumed native grasses supplemented with contaminated corn kernels and wheat bran at a concentration of 12.5 mg FB1/kg and 5.3 mg FB2/kg had very clear signs of ELEM [143].

Ross and Collar demonstrated that FB2 at a concentration of 75 mg/kg diet was able to induce hepatitis and ELEM lesions in ponies after 150 days of exposure. By contrast, FB3 at concentration 75 mg/kg of diet was unable to induce any effect on serum enzymes, clinical signs and histopathology changes in ponies after 57 to 65 days of exposure [111]. Few years later, Riley and Showker conducted the same experiment on ponies with the same dose of 75 mg FB2/kg diet for 136 - 223 days, or 75 mg FB3/kg diet for 57 - 65 days. After 48 days, ponies fed with FB2 had an increase in serum enzymes of liver toxicity and clinical signs (head shaking, gait problems, and muscle tremors), whereas ponies fed with the FB3 diets did not show any increase in serum enzymes or clinical signs for as long as 65 days . Disruption of sphingolipids metabolism was obtained 4 and 11 days after FB2 and FB3 exposure, respectively [112]. Thus, it was suggested that FB3 was less toxic than FB2 or FB1 in equine.

The nature and intensity of the symptoms observed were very variable, and they do not show a specific direct relationship between the importance of the clinical signs and the degree of the cerebral lesion. The clinical signs which have been observed due to nervous disorders are: hyperesthesia, hyper-excitability, ataxia, euthanasia, trembling, reluctance to move, walk in a circle, push the wall, and fall on one side, paresis of the lower lip and tongue, and inability to eat or drink [113-144-145]. Depression, paralyses and jaundice symptoms are also linked to the disease [4 -

111]. Death can be sudden or preceded by convulsions and a state of coma. In all cases, death is expected and occurs within a few hours or a few days after the onset of the disorders [105-109-114]. Among autopsy findings, the main one observed was necrosis in brain white matter, brain stem, cerebellum, and spinal cord. Hemorrhages in CNS and abdominal cavities, edematous brain and perivascular hemorrhage were occasionally present [9-115].

2.1.1.2. Hepatotoxicosis

Hepatotoxicosis is a fatal disease produced by consumption of high quantity of FB1, while exposures to lower quantity of toxin probably produce ELEM. The dose limit between the hepatotoxicosis and ELEM is not clear. Hepatotoxicosis and ELEM are accompanied by lesions on the nervous system and hepatic modifications [115]. Cases of hepatotoxic syndrome are less frequent than those of the neurotoxic form, and death often occurs within 5–10 days of clinical signs onset. Global clinical symptoms of hepatotoxicosis are loss of appetite and depression followed by oedema of the head and a clear icterus. More specific markers are an increase in serum bilirubin and liver enzyme activities. At a necropsy investigation, liver becomes solid, yellow and small in size [168].

Hepatositis was observed in an experiment conducted on two horses after feeding them cultivated corn with *Fusarium moniliforme* MRC 826 through a stomach tube. One horse developed severe hepatosis and mild edema of the brain after 6 doses of 2.5 g of culture material/kg b.w. /day in 7 days. The second horse, which received a half dosage of 1.25 g/kg b.w. /day, developed mild hepatosis and moderate oedema of the brain [253].

In another study, hepatic necrosis and mild encephalopathy were observed in ponies receiving 44 mg FB1/kg feed, for 9 - 45 days. While hepatic necrosis accompanied by ELEM was found in the animals treated with a high dose of 88 mg FB1/kg feed for 75 - 78 days by oral route [116-252].

2.1.1.3. Cardiotoxicity

Evidence of cardiovascular dysfunction was detected after neurologic symptoms appeared in horses receiving daily IV injections of 0.01, or 0.20 mg FB1/kg

b.w. for 7 to 28 days. That evidence was represented by a decrease in heart rate, cardiac output, right ventricular contractility and coccygeal artery pulse pressure. Alteration of sphingolipids metabolism in serum and myocardial were observed in all animals treated. The NOEL for cardiovascular abnormalities was 0.2 mg/kg b.w. per day, but the NOEL for serum biochemical abnormalities was less than 0.2 mg/kg b.w. per day, [81-150-168-169].

2.1.2. Swine

Fumonisin toxicosis in swine is characterized by injury to pulmonary, hepatic, cardiovascular, and immune systems as well as alteration of sphingolipids metabolism and effects on growth rate [119].

2.1.2.1. Porcine pulmonary edema (PPE)

In 1981, it was the first time that pulmonary edema was induced in experimental swine fed with corn contaminated with *F. verticillioides* [118]. In 1989, thousands of pigs died from pulmonary edema after ingesting corn contaminated by fumonisins in the mid-western and south-eastern parts of the United States. Following autopsy it was established that the cause of death was related to pulmonary oedema and hydrothorax with the thorax cavity filled by a yellow liquid. Feed samples were taken from the outbreak areas, and the presence of detected FB1 was in the range of 20-330 mg/kg of feed. It was then that the disease was named Porcine Pulmonary Edema Syndrome (PPE) [21-120]. This disease was linked to ELEM by means of having the same causative agent [129]. Many studies were conducted by using contaminated feed and purified toxin to confirm the disease. The results obtained from those studies demonstrated that pulmonary edema in pigs was recorded only with high levels of FB1, while animals fed with low levels of FB1 suffered from hepatotoxicosis as will be explained later the section of swine [21].

Lethal pulmonary oedema was observed within 4-7 days after consumption of feed contaminated with FB1 at concentrations of higher than 16 mg/kg b.w. /day [21-121-122-123-124]. Similarly, lethal pulmonary edema was obtained after 5 days in pigs which received daily high dose of 0.4 mg FB1/kg b.w. by intravenous route. Meanwhile, pigs which received daily low dose at concentration 0.174 mg FB1/kg

b.w. for 7 days by intravenous route did not develop pulmonary edema [121]. Concurrently, weaning pigs fed lower doses of FB1 in a culture material (10–40 mg/kg of diets) for 4 weeks did not show any clinical signs of toxicity [125].

Weaned piglets had a chronic oral exposure to contaminated diets at levels 0, 10, 30 mg of FB1/kg of feed for 28 days. All signs of toxicity were only localized in the highest dose group, which was characterized by a decrease in feed consumption and body weight gain, an increase in organ weight (lung, liver and heart), an increase in hematological and biochemical parameters. After 20 days of feeding animals with high dose (30 mg of FB1/kg diet) the typical clinical signs of pulmonary edema were appeared, such as, cyanosis of ears, tail, eyes sclera and mucosal membranes, increased heart and respiratory rate with shallow breathing [159].

Pulmonary edema was also observed in piglets at one week of age after feeding pregnant sows with high dose 300 mg FB1/kg of diets during the last week of gestation and the first week after parturition. That means, possibility of FB1 to cross sow placenta and excreted in its milk is possible [126].

Clinical signs in the twelve hours preceding development of pulmonary edema and death are: inactivity, increased respiratory rate, and decreased heart rate [127]. During the dying hours animals show more exhausted respiratory distress, increased respiratory rate and effort with abdominal and open mouth breathing [122,128]. Autopsy finding are mainly localized in severe pulmonary oedema, hydrothorax and perivascular oedema [21-25].

FB1 decreases cardiac contraction, mean systemic arterial pressure, heart rate and cardiac output. At the same time, FB1 increases mean pulmonary artery pressure and pulmonary artery wedge pressure [130-131]. On the other hand, FB1 inhibits L-type calcium channels by modification of sphingolipids metabolism in the heart. All of those events lead to acute left-sided heart failure, which is considered the first cause of pulmonary edema [127]. More recent studies have implied that other causes of pulmonary oedema are a consequence of vascular alterations due modification of sphingolipids metabolism after exposure to FB1 in vitro [132].

2.1.2.2. Hepatic injury (hepatotoxicosis)

Hepatic injury is dependent on the dose and exposure time to a toxin. That means the toxic dose necessary to induce hepatic injury is lower than the toxic dose necessary to induce pulmonary oedema. This hypothesis was defended by a study conducted on pigs fed with different concentrations of FB1. The results demonstrated that only hepatic lesions without clinical of pulmonary oedema were obtained in group fed on a diet containing less than 4 mg of FB1/kg b.w. /day by oral route for 2 weeks. Whereas, pulmonary oedema and hepatotoxicosis were observed in pigs which had consumed diets containing 4.5 and 6.3 mg of FB1/kg b.w. /day for 2 weeks [122, 125, 130].

2.1.2.3. Immunosuppression effect of fumonisin in pigs

In vitro, incubation of alveolar macrophages with 2, 5, 10 and 25g FB1/ml, for 72 hours decreased production of interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) at all dose levels after 24 hours of incubation. Whereas, FB1 at concentrations of 5 and 25 g/ml, for 72 hours of incubation was able to reduce the number of alveolar macrophages viability down to 65 and 45%, respectively, comparing to control levels [232].

In vivo, weaning piglets were fed contaminated diet of 8 mg FB1/kg of feed, for 28 days. The animals were vaccinated with inactivated mycoplasma agalactiae at the 8th and 22nd days of the experiment. Results obtained demonstrated that FB1 induced a significant decrease in IL-4 expression in the blood. On the other hand, FB1 had no effect on serum concentration of the immunoglobulin subset (IgG, IgA, and IgM) [233].

A new study was conducted in 2012 on piglets treated with purified 2.8 μ mol FB1 or HFB1/kg b.w. /day, by gavage, for 2 weeks. The FB1 treated group had a significant increase in liver IL-1 β and IL-8, and a significant decrease in IL-2, IL-6, INF α and INF γ of liver and small intestine lymph nodes. Conversely, the HFB1 treated group showed only a slight decrease in IL-6 in liver and small intestine lymph nodes. Hence, it was deduced that FB1 has a stronger immunosuppressive ability when compared to HFB1 [166].

2.2. Low sensitivity species

Ruminants

Cattle are less sensitive to FB1 toxicity when compared to horses and pigs. Ruminant resistance to FB1 toxicity is an outcome of their lower bioavailability ($F = 0.5 - 1\%$ of oral dose), and ability of their micro-flora to degradation FB1 to hydrolyzed forms (HFB1/2 or AP1), as it was explained earlier in fumonisin pharmacokinetics chapter (absorption and metabolism sections)

Two Jersey cows ingested contaminated feed at a concentration of 75 mg FB1/kg (equivalent to 3 mg FB1/kg b.w. /day) for 14 days. The results did not demonstrate any important sign of toxicity. Transient diarrhea was obtained at the start of the feeding program, as well as an increase in serum cholesterol concentration and decreased feed intake and milk production [137].

An experiment carried out on eighteen feeder calves which were fed contaminated diets with mixture fumonisins (FB1, FB2, and FB3) at total ranges: 5, 31 and 148 mg/kg for 31 days. No effect was observed at all concentrations relative to average feed intake and body weight gains. Slight loss of appetite was recorded in calves fed with the high dose of the contaminated diet. Only some biochemical parameters were modified in two of the calves which had consumed 148 mg/kg of feed after 10 days of exposure [133]. Autopsy revealed that gastrohepatic lymph nodes of animals that had received high FB1 doses were mildly enlarged, oedematous and contained petechial hemorrhages. These lesions were not specific, and there are no other reports available on cattle for comparison. By contrast, gastrohepatic lymph nodes of control animals were not affected. Moreover, under microscopic lesion examination, mild hydropic degeneration and cloudy swelling were observed in the livers of animals fed highly contaminated diet, but not in control animals [133].

Long term experiments conducted on five Holstein steers which consumed mildly contaminated diet with an average of 94 mg FB1/kg of feed daily for 253 days. No clinical signs or either gross lesions were obtained at the end of study. Only some biochemical parameters were significantly increased in serum of the treated

group. There was presence of mild histological evidence to hepatocellular injury and biliary epithelial hyperplasia [138].

Milk-fed calves were treated with 1 mg FB1/kg b.w. daily for 7 days by intravenous route. On the fourth day of treatment, loss of appetite and anorexia were observed. Evidence of sever hepatic and bile duct injury were detected by an increase of serum activity of some enzymes after 4 days of treatment [139]. Renal functions injury was diagnosed by an increase of some biochemical biomarkers in the serum, and by urine specific gravity at the end of the experiment [139].

Lambs were injected intra-uminally with culture material in different doses of 11.1, 22.2 or 45.5 mg (FB1, FB2 and FB3)/kg b.w., for 4 days. Severe nephrosis and hepatosis were the main consequences of a fumonisins exposure at high dose in sheep. Theses damages were indicated by changes of biochemistry in the dosed lambs serum. Liver and kidney damages were confirmed at the end of study by histological examinations which detected renal tubular necrosis and mild hepatopathy [140]. These results harmonized with previous data obtained in 1981 by Kriek NP [141].

Similar effects of FB1 in sheep were obtained in weaning Angora goats after consumption of feed containing 95 mg FB1/kg of feed, for 112 days. Liver damage and kidney dysfunction enzymes were elevated in the blood. [142].

3. Farm animals: poultry

In general, poultry is less sensitive to FB1 toxicity when compared to pigs and horses. Poultry are classified according to their sensitivity to FB1 exposure as follows:

3.1. Low sensitivity species (layer hens and broiler)

A long term experiment was conducted on laying hens at 24 weeks of age, fed on 100 or 200 mg FB1/kg of feed for 420 days. No effects on body weight with significant decrease in egg production were observed at both concentrations during the study. Increased egg weight was observed in hens fed the 100 mg FB1/kg diet at the end of the egg production cycle (252 days of egg production). In general no significant mortality was detected during the 420 days of the experiment. One death case was recorded each in the control group and the treatment group with 200 mg

FB1/kg. Furthermore, there were four death cases due to uterine prolapses, which may be attributed to the increased egg weights in the treatment group with 100 mg FB1/kg. After 112 days, the group fed the highest contaminated level 200 mg FB1/kg feed had some modification of serum biochemistry (table 10) [155].

Table 10: Adverse effects of FB1 on chickens

Dose and duration	Descriptions	Ref
Laying hens 100, 200 mg FB1/kg feed/ 420 days	No mortality, no BW decrease Weak effect on biochemistry Decrease egg production. Weak egg weight increase.	[155]
Broiler 20- 80 mg FB1/kg feed/ 21 days	No signs of toxicity No effect on biochemistry Alteration of sphingolipids	[146]
Broiler 125, 274 mg FB1/kg feed/ 14 days	Increased mortality (20 - 50%) Young more sensitive	[147]
Broiler 100-400 mg FB1/kg feed/ 21 days	400 mg/kg decrease BW and increased body organ weights Necrotic hepatic foci Altered biochemistry	[148]
Broiler 33 - 627 mg FBs/kg feed/ 21 days	Reduction in weight gain and feed conversion Hepatocellular hyperplasia	[150]
Broiler 75- 525 mg FB1/kg feed/ 21 days	> 450 mg/kg : decrease feed intake, BW gains, increase liver and kidney weights. > 150 mg/kg Hepatocellular hyperplasia All doses: alteration of sphingolipids	[151]
Broiler 50-200 mg FB1/kg feed/ 21 days with infectious challenge	200 mg /kg: decrease lymphocyte proliferation enhance bacterial colonies in blood, spleen, and liver. decrease secondary antibody response.	[152]
Broiler 25, 50 mg FB1/kg feed/ 42 days	No mortality, no BW decrease Unexpected effects on biochemistry All doses: alteration of sphingolipids in liver	[153]

Broiler chickens of one day age were fed purified FB1 at concentrations 0, 20, 40, and 80 mg/kg feed for 21 days. No signs of toxicity such as decrease of body weight and growth rate were observed. In liver, a significant disruption of sphingolipids metabolism was detected in all treated groups, whereas, it appeared in

serum only in the group fed with 80 mg FB1 /kg. Biochemistry was not affected in chickens fed the highest FB1 level 80 mg/kg, (table 10) [146].

Reduced weight gain was observed in one-day old male broiler chickens exposed to high doses of purified FB1, either 125 or 274 mg FB1/kg of feed for 14 days. The mortality percentages with the low and high contaminated diets were 20% and 50%, respectively. The spontaneous death is only observed during the first three days of life, which illustrated that younger chickens were more sensitive to FB1 toxicity than older chickens, (table 10) [147].

One day-old chicks were fed diets containing 0, 100, 200, 300, or 400 mg FB1/kg of feed, for 21 days. After 4 days of treatment bird excreta became dark, sticky and adhered around the vent area of the birds. These symptoms declined after 13 days and excreta become normal after 21 days. Body weight gain was not affected in the first week, but a 20% decline was recorded with the highest level of FB1 in the second week of the experiment. Also, at the end of the experiment, the weight of the liver, proventriculus and gizzards increased. Whereas no significant effects were obtained concerning the weight of the kidneys, heart, spleen, pancreas, and the bursa of fabricius. In histological examinations, only in chicks receiving ≥ 100 mg FB1 /kg hepatocellular hyperplasia and small multiple foci of hepatic necrosis appeared. Necrotic foci were consistent in size at all dosages, but were present in greater numbers in chicks fed 300 or 400 mg FB1/kg. Biliary epithelial hyperplasia was observed in the livers of chicks fed 300 or 400 mg FB1/kg. Some serum biochemical parameters increased at higher FB1 levels (table 10) [148].

One-day old chickens consumed *F. moniliforme* culture material containing total amounts of fumonisins (FB1, FB2 and FB3) at concentrations of 33, 99, 132, 330, 429 and 627 mg/kg of feed, for 21 days. Weight gain and feed conversion were reduced depending on the dose of toxin exposure. Slight hepatocellular hyperplasia was obtained at concentrations 99 to 132 mg/kg, while moderate hepatocellular hyperplasia was detected at concentration 330 mg/kg, and with severe hyperplasia at the highest contaminated diets 429 and 627 mg/kg, (table 10) [150].

One-day old chicks were fed a diet treated with FB1 at levels 0, 75, 150, 225, 300, 375, 450, and 525 mg/kg of feed, for 21 days. Birds which consumed the highest toxin level at concentrations of 450 and 525 mg FB1/kg of diet had a decrease in feed

intake and body weight gain, increased liver and kidney weight, increased mean cell haemoglobin concentrations compared to the control group. Hepatocellular hyperplasia was observed in chicks fed diets equal to or more than 225 mg FB1/kg of diet. Disruption of sphingolipids metabolism was observed at all concentrations of contaminated diets. Thus, it was suggested that diets containing 75 mg FB1/kg of feed may be toxic to young broiler chicks, (table 10) [151].

Three experiments with one-day old broilers were conducted to measure immunosuppression in chicks. Experiment (1) - Chickens were give free feed access at concentrations of 50, 100 or 200 mg FB1/kg of feed for 3 weeks and were injected intravenously with $4.6 \times (10 \times 6)$ *Escherichia coli* for 21 days. Experiment (2) - Chickens were fed 50, 100 or 200 mg FB1/kg of feed for 4 weeks, and then were injected with 0.5 ml inactivated Newcastle Disease vaccine on weeks 2 and 3 of the experiment. Primary and secondary antibody titrations were measured at 7 days after each injection. Experiments (3) - Chickens were fed 200 mg FB1/kg of feed for 3 weeks, without any supplements. The results obtained in the first experiment showed that chicks with the highest concentrated feed 200 mg FB1/kg diet have higher significant numbers of bacterial colonies in blood, spleen and liver when compared to the control group. The second experiment results recorded significant decrease in titration of secondary antibody response with high contaminated feed 200 mg FB1/kg. The results obtained in the third experiment showed a significant decrease of lymphocyte proliferation in chicken fed with 200 mg FB1/kg when compared to the control group, (table 10) [152].

In 1-week old broilers were fed culture contaminated diets 0, 25, or 50 mg FB1/kg of diet, for 42 days. Feed intake, body weight gain, and feed conversion of chicks were not affected. No significant differences between control and treated groups were observed in the weight of the liver, heart, kidneys, pancreas, bursa of fabricius, gizzards or spleen. Except, chicks fed 25 mg FB1/kg which showed a significant decrease in proventriculus weight compared to control groups or the highest dosage 50 mg FB1/kg group. The results from the serum biochemistry analysis showed that chicks fed 50 mg FB1/kg had decreased serum calcium and increased serum chloride levels. No hematological effects were obtained relative to hemoglobin, RBC counts, hematocrit, MCV, MCH, or MCHC. Liver disruption of

sphingolipids metabolism was observed at all concentrations of contaminated diets, (table 10) [153].

3.2. Mild sensitivity (turkeys)

One day- turkey poultts were fed contaminated diets at levels of 75, 150, 225, or 300 mg FB1/kg of feed for 21 days from culture material (FCM). The lowest concentration corresponded to a daily dose of approximately 9 mg FB1/kg b.w. on average. Decrease in feed intake and body-weight gain and increase in liver weight were dependent on the exposure dosage. Poultts fed low contaminated diet 75 mg FB1/kg consumed more feed than other groups. Therefore, they had pronounced lower body-weight gains than controls or poultts fed high contaminated diet. Alteration of sphingolipids in serum and hepatocellular hyperplasia lesions were detected in all FB1 treatment groups, whereas biliary hyperplasia was established in poultts fed 150 to 300 mg FB1/kg, (table 11) [154].

Table 11: Adverse effects of FB1 on turkeys

Dose and duration	Descriptions	Ref
75-300 mg FB1/kg feed/ 21 days	Decrease feed intake , BW gain Increase in liver weight, hepatocellular, biliary hyperplasia >75 mg/kg: alteration sphingolipids serum	[154]
75 mg FB1/kg feed/126 days	Decrease BW gain Increase liver weight	[161]
25-475 mg FB1/kg feed/ 21 days	Dose depending hepatocellular hyperplasia >250 mg /kg: decrease feed intakes and B.W. gains ≥ 175 mg /kg increase liver, pancreas weight >325 mg/kg ateration of biochemistry >25 mg/kg: alteration sphingolipids liver	[149]
25, 50 mg FB1/kg feed/91 days	50 mg /kg: decrease feed intake	[153]
5-20 mg FB1+FB2/kg feed/63 days	>5 mg/kg: alteration sphingolipids liver and kidney	[156]

A chronic toxicity study was conducted on one-day old turkeys fed balanced rations containing 75 mg FB1/kg of feed, for 18 weeks. Nil mortality was noted. Decrease in body weight gain was demonstrated on weeks 4, 10, and 12 of experimental. At the end of the treatment the liver had become significantly heavier, (table 11) [161].

One-day old turkey poults were selected randomly for dietary treatments containing 0, 25, 50, 75, 100, 175, 250, 325, 400, and 475 mg FB1/kg of feed, for 21 days. Poults fed 325 to 475 mg FB1/kg diet had a significant decline in feed intake and body weight gain. Increased liver and pancreas weights were observed in poults fed ≥ 175 mg FB1/kg. Increase in red blood cell counts and serum biochemical parameters appeared in birds fed 400 and 475 mg FB1/kg of diet. Hepatocellular hyperplasia was mild at 75 and 100 mg FB1/kg diet, moderate to severe at 250 mg FB1/kg, and severe at 325 to 475 mg FB1/kg. Liver alteration of sphingolipids increased at all diet doses. These results indicated that diets containing ≥ 75 mg FB1 /kg are toxic to young turkeys, (table 11) [149].

In 2002, Broomhead conducted a study to evaluate the chronic effects of FB1 by applying doses lower than the ones recommended by US-FDA (2001). one week old broiler chicks and turkey poults, were fed culture contaminated diets 0, 25, or 50 mg FB1/kg of diet, for 42 days in chicks, and a 90 days in turkeys. Feed intake, body weight gain, and feed conversion of chicks were not affected by consumption of FB1. Whereas, Turkeys fed 50 mg FB1/kg had significantly lower feed intake than the controls. No significant differences were observed in the weight of internal organs in both species. Except, chicks fed 25 mg FB1/kg had a significant decrease in proventriculus weight compared to control groups. No haematological effects were obtained in both species relative to haemoglobin, RBC counts, hematocrit, MCV, MCH, or MCHC. Liver alteration of sphingolipids was significantly increased in both species at all doses. Results indicate that 50 mg FB1/kg diet is harmful to turkeys but it is not toxic to broilers fed to market age, (table 10 and 11) [153].

The first study which investigated the maximum level of fumonisins in avian feed recommended by the European Union (2006) was conducted on turkeys after exposing them to contaminated diets with 0, 5, 10, and 20 mg of FB1+FB2/kg of feed, over a period of 9 weeks. The results obtained indicate that no sign of toxicity and no effects were observed concerning turkey general performance, feed consumption, growth and weight of tissues at all level of fumonisins in the feed. Fumonisins did not induce a disturbance on biochemical parameters with any level of fumonisins. Marked modifications of sphingolipids metabolism in liver and kidney were

recorded for all doses. For the first time it was demonstrated in turkeys, that alteration of sphingolipids occurred a few days after exposure to fumonisins at a level of 20 mg of FB1 + FB2/ kg of feed. Finally, this study confirmed that the level of fumonisins in avian feed of 20 mg of FB1 + FB2/kg recommended by the European Commission could be considered as safe for these animals. However, the lack of effect of fumonisins on performance and on biochemistry does not mean that these mycotoxins have no effect on turkeys (table 11) [156].

3.3. High sensitivity (ducks)

Pekin ducklings were fed rations containing 100, 200 or 400 mg FB1/kg feed, for 21 days. Mortality was observed only in ducks fed high dose 400 mg FB1/kg feed, two out of eight treated cases died. Additionally, the results exposed that a decrease in feed intake and weight gain and an increase in the weight of the liver, heart, kidneys, and proventriculus were dose-dependent. An alteration of sphingolipids in liver was observed at all contaminated diets levels. Histopathology investigation revealed hepatocellular hyperplasia in all ducklings fed FB1, and mild to moderate biliary hyperplasia was obtained only at the highest toxic dose, (table 12) [162].

Table 12: Adverse effects of FB1 on ducks

Dose and duration	Descriptions	Ref
100- 400 mg FB1/kg feed/21 days	400 mg/kg: increase mortality >100 mg/kg: decrease feed intake, B.W. gain >100 mg/kg: increase organ weights, hepatocellular hyperplasia >100 mg/kg: alteration sphingolipids.	[162]
5- 45 mg FB1/kg b.w. /12 days.	>5 mg/kg: Increase liver weight All doses: alteration of biochemistry and sphingolipids serum.	[163-164]
2- 128 mg FB1/kg feed/ 77 days.	>8 mg/kg: decrease BW, increase organs weight. >8 mg/kg: alteration of biochemistry All doses: alteration of sphingolipids (liver, kidney, serum)	[165-170-171]
10, 20 mg FB1/kg feed/ 12 days.	20 mg/kg: increase mortality All doses: alteration of sphingolipids liver, serum	[158]

Mallard ducks received 0, 5, 15 or 45 mg FB1/kg b.w., daily by force-feeding, over 12 days. No signs of toxicity were observed relative to mortality or decreased body weight. Increased weight of liver was dose-dependent. Hepatic biochemistry

was modified at all doses. By contrast, kidneys biochemistry was not affected. The above results concluded that liver was more affected to FB1 toxicity than kidneys. Sphingolipids were modified in serum at all doses after six days of treatment. Only two days with ≥ 5 mg FB1/kg of diets were necessary to induce sphingolipids modifications in serum, (table 12) [163-164].

Seven-day old male Mallard ducks were exposed by oral route to purified FB1 at different levels of 0, 2, 8, 32, and 128 mg of FB1/kg of feed, for 77 days. No mortality was observed in any of the treated groups. Feed consumption and body weight gain were not affected at the end of experimental. Decreased body weight was observed in groups which ingested feed at levels of 32 and 128 mg/kg, from day 28 to 63 and from day 7 to 63, respectively. Ducks which had received 32 and 128 mg FB1/kg had increases in the weights of their gizzards, spleens, and livers. Some serum biochemical parameters were elevated after one week of exposure to mild and high contaminated diets. Other ones were not affected at any dosage. Alterations of sphingolipids metabolism in serum, liver, and kidney were established at all diet levels from 2 to 128 mg of FB1/kg of feed, and the highest disruption value was recorded in the kidneys. Thus, it was suggested that the kidney is the most sensitive organ to FB1 exposure. The lowest investigated dose which could increase Sa and Sa:So ratio in liver and kidneys after 7 days of exposure was 2 mg/kg of feed, (table 12) [165-170-171].

Mallard ducks at 12 weeks of age received by force-feeding for 12 days, contaminated maize at the maximum level permitted by the European Union - 0, 10 and 20 mg FB1/kg of feed. That corresponded to a final average feed intake of approximately 10 kg of maize per duck. 8% mortality was observed only in ducks fed 20 mg of FB1/kg of feed. It was difficult to identify FB1 effects on the liver weight and serum biochemical parameters, because they normally increase with force-feeding. All FB1 treated groups had disruption of sphingolipids metabolism in the liver and serum. Force-feeding did not create any alteration in sphingolipids, (table 12). Thus, sphingolipids alteration could be used as the best parameter to FB1 exposure [158].

3.4. Conclusion

Fumonisin B1 induces a spontaneous disease in horses and pigs while cattle and poultry are much more resistant. Horses show more susceptibility to FB1 toxicity than other animal species. FB1 attacks specific species and specific target organs. For example, the target organ in horses is the brain where equine leukoencephalomalacia (ELEM) is induced, while the target organs in pigs are the lungs and porcine pulmonary oedema (PPE) is induced. These differences impose specific recommendations concerning the tolerable levels of fumonisins in feed.

Concerning the avian species, strong differences seems observed depending on the age and species. FB1 induces mortality in ducks fed low dose of FB1 (20 mg/kg of feed), and in young broilers fed high dose of FB1 (≥ 125 mg/kg of feed), for few days. By contrast, no mortality is recorded in turkeys or old broilers and laying hens exposed to high doses of FB1 (≥ 200 mg FB1/kg feed), for several weeks or months. Hence, it is suggested that ducks and younger broilers were more sensitive to FB1 toxicity than older broilers, and other poultry species. However, avian species are often taken as a homogeneous group in terms of toxicity and recommendations (see chapter IV)

Liver and kidneys are target organs of fumonisins toxicity in all animal species. FB1 increases hepatotoxicity and biochemistry in all species. Alteration of sphingolipids metabolism is often observed before signs of toxicity. This observation and the structural analogy between fumonisins and sphingolipids make of interest the specific analysis of the consequences of FBs exposures on sphingolipids metabolism, and the relation between these alteration and organs toxicity. In addition, FB1 has adverse effects on the immune function system which can increase susceptibility to opportunistic micro-organisms invasions.

4. Humans

No data was available concerning acute effects of FB1 toxicity on humans, although, FB1 has been reported at high concentrations (118 –155 mg/kg of food) from home grown maize in South Africa and China [77]. High incidence of esophageal cancer observed in human populations of Transkei in South Africa, and Henan Province in China was correlated with high intake of maize contaminated by FB1. By contrast, highest mortality rates for oral pharyngeal in Italy and esophageal cancer in Europe were not clearly correlated with consumption of contaminated maize with FB1[77]. IARC (International Agency for Research on Cancer) classified FB1 and FB2 as carcinogenic derived class 2B [267].

A number of estimates of human exposure to fumonisins have been made in different countries, such as in the USA: 0.08 µg/kg b.w. per day; in Canada: 0.017–0.089 µg/kg b.w. per day; in Switzerland: 0.03 µg/kg b.w per day; and in the Transkei of South Africa: 1.2 to 355 µg/kg b.w, per day. Thence, exposure to FBs in human is generally bellow than the recommended PMTDI of 2 µg/kg b.w./day estimated for fumonisins (page 34).

IV. Fumonisin recommendations

Fumonisin toxicosis are linked to certain diseases in several animal species and in human, such as pulmonary edema pigs [11-12], encephalomalacia in horses [9], hepatic and renal toxicities in equines, pigs, sheep, rodents and poultry [15-16-17] and esophageal cancer in humans [4-21-77]. Furthermore, diagnosis of fumonisin toxicosis is difficult, which based on clinical signs, histopathology examination, and the presence of fumonisins at toxic levels in the feed. In addition, there is not specific treatment or antidote to fumonisin toxicosis. In order to solve these problems, the European Union and FDA have issued guidance levels for total fumonisins in human and animal feed to avoid fumonisin toxicosis (table 13, 14 and 15) [25-157-160-267].

Table 13: European recommendation maximum levels for FBs in animals feed [157]

Mycotoxins	Products intended for animal feed	mg/kg of feed
FB1 + B2	maize and maize products	60
	pigs, horses (Equines), rabbits and pet animals	5
	Fish	10
	poultry, calves (< 4 months), lambs and kids	20
	adult ruminants (> 4 months) and mink	50

Table 14: FDA-recommended maximum levels for FBs in human foods [160]

PRODUCT	TOTAL FUMONISINS (FB1 + FB2 + FB3 ng / g)
Dry milled corn products (e.g., flaking grits, corn grits, corn meal, corn flour with fat content of < 2.25%, dry weight basis)	2000
Whole or partially dry milled corn products (e.g. flaking grits, corn grits, corn meal, corn flour with fat content of ≥ 2.25 %, dry weight basis)	4000
Dry milled corn bran	4000
Cleaned corn intended for mass production	4000
Cleaned corn intended for popcorn	3000

Table 15: FDA^a guidance levels for total FBs in animal feed [25-267]

Animals or class	Recommended maximum level of total fumonisins in corn and corn by-products (ppm; mg/kg)	Recommended maximum level of total fumonisins in total ration (ppm; mg/kg)
Horse ^b	5	1
Rabbit	5	1
Catfish	20	10
Swine	20	10
Ruminants ^c	60	30
Poultry ^d	100	50
Ruminant, poultry and mink breeding stock ^e	30	15
All others ^f	10	5

(a) From Food and Drug Administration (2001). (b) Includes asses, zebras, and onagers. (c) Cattle, sheep, goats, and other ruminants that are ≥ 3 months old and fed for slaughter. (d) Turkeys, chickens, ducks, and other poultry fed for slaughter. (e) Includes laying hens, roosters, lactating dairy cows and bulls. (f) Including dogs and cats.

V. Fumonisin mechanism of actions

1. Sphingolipids metabolism

Fumonisin is structurally similar to sphinganine (Sa) and sphingosine (So) (figure 2). Thence, fumonisin interferes with sphingolipids biosynthesis by blocking the ceramide synthase enzyme [25]. The primary biological consequences to ceramide synthase enzyme blockage are inhibition of sphingolipids synthesis (sphingosine, ceramide and complex sphingolipids), and increase concentration of free sphingolipids and Sa:So ratio in the tissue and serum of exposed animals, or in cell cultures [175].

The concentration of free sphingolipids (sphingosine, sphinganine and ceramide) and sphingolipids phosphorylated forms (So-1P, Sa-1P and C1P) play an important role in determining cell fate. Given that, they have negative effects on body cells [183-187]. Any disturbance in the sphingolipids metabolism balance or rheostat balance can lead to serious disorders, such as neurodegenerative diseases (Alzheimer or Parkinson), cardiovascular diseases, chronic inflammation (asthma), or cancer [179-197]. In addition, disturbance of the sphingolipids metabolism is combined with fumonisin toxicity in different animal species, such as ELEM in horses, PPE in pigs, as well as hepatic and renal toxicity in all animal species [9-10-11-12-15-16-17-205].

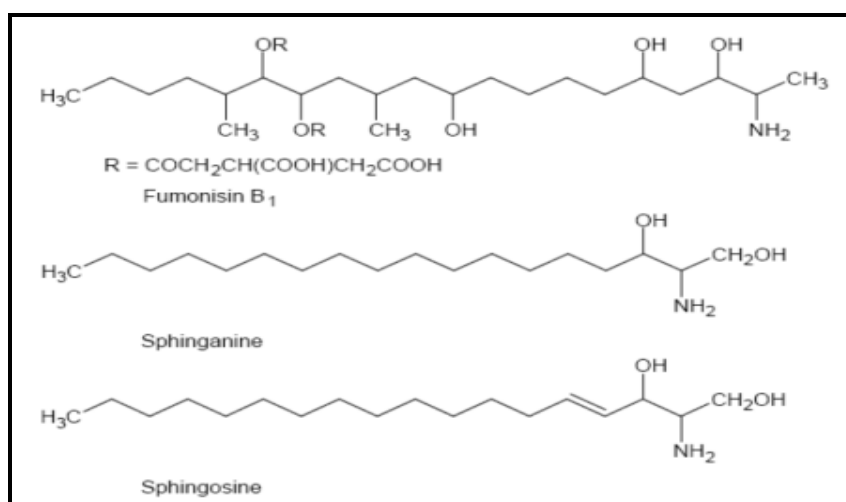


Figure 2: Structural analogy of FB1 and sphinganine and sphingosine [25]

1.1. Historical background of sphingolipids

In 1884, Johann L. W. Thudichum discovered some unknown molecules from brain tissue extracts. These molecules were named after the Sphinx. Few years later, it was considered that sphingolipids play primarily structural roles in the cell membrane formation. The main lipids of the cell membrane are glycerolipids, phospholipids and cholesterol [173-174-175]. Sphingolipids are principally found in all mammalian cell membranes, particularly nerve cells and brain tissue. At the end of the twentieth century, it was reported that sphingolipids molecules, in particular ceramide, play important roles in signal transmission and cell recognition that control cell growth, differentiation, and apoptosis. Cell membrane sphingolipids are amphipathic molecules that have both hydrophobic and hydrophilic properties. The hydrophobic molecules include sphingosine and sphinganine. In contrast, hydrophilic molecules include phosphate groups [sphingosine-1-phosphate (So-1P) and ceramide-1-phosphate (C1P)], sugar residues groups in glycosphingolipids (GSLs), and OH groups in ceramide [176]. Sphingolipids are derivatives of lipid sphingosine characterized by a long chain consisting of approximately 14 to 20 carbons in length, with an amino group at position 2 and hydroxyl- at positions 1 and 3 [174]. Sphingolipids are classified as simple sphingolipids (sphingosine, sphinganine and ceramide) and complex sphingolipids [sphingomyelin and glycosphingolipids (glucosylceramide and galactosylceramide) [173-175-179].

1.2. Sphingolipids metabolism (synthesis and catabolism)

1.2.1. Sphingolipids synthesis pathway

Synthesis of several sphingolipids, such as sphingosine (So), sphinganine (Sa) and ceramide (Cer) occurs in the endoplasmic reticulum (ER). Meanwhile, synthesis of sphingomyelin (SM) and glycosphingolipids (GSLs) occurs in the Golgi apparatus after transporting ceramide from ER to the Golgi apparatus [180-181]. Biosynthesis of sphingolipids is initiated in the ER by condensation and transformation of serine and palmitoyl-CoA to 3-keto-sphinganine, which catalyzed by serine palmitoyl transferase enzyme (SPT). 3-keto-sphinganine is subsequently reduced to sphinganine (dihydro-sphingosine) by 3-keto-sphinganine reductase (3KSR).

Sphinganine is produced through a biosynthetic pathway, whereas the main quantity of sphingosine is produced by sphingolipids degeneration pathway. Ceramide synthase (CerS) acylates sphinganine to dihydroceramide (DHCer), which is converted to ceramide by dihydroceramide desaturase (DHCD). Ceramide is transported from ER to the Golgi apparatus by a transfer protein (CERT). There ceramide is transformed to sphingomyelin via sphingomyelin synthase (SMS), or to glucosphingolipids such as galactosyl-ceramide and glucosyl-ceramide, by using galactosyltransferase (CGaIT) and glucosyl-ceramide synthase (GCS), respectively, (figure 3) [178-180-181-203].

1.2.2. Sphingolipids degeneration pathway

All steps of sphingolipids degeneration take place inside the ER [203]. Sphingolipid ceramide might hydrolyze to sphingosine (So) in the presence of ceramidase enzyme. Sphingosine is recycled to ceramide by using ceramide synthase (CerS). In parallel, free sphingolipids, such as sphinganine, sphingosine and ceramide are able to phosphorylate by kinase enzymes to generate sphinganine one phosphate (Sa-1P), sphingosine one phosphate (So-1P) and ceramide one phosphate (C1P), respectively. After that, sphinganine and sphingosine phosphorylated forms are hydrolyzed to ethanolamine phosphate and palmitic aldehyde by the phosphatase enzyme, (figure 3) [179-181-182-203].

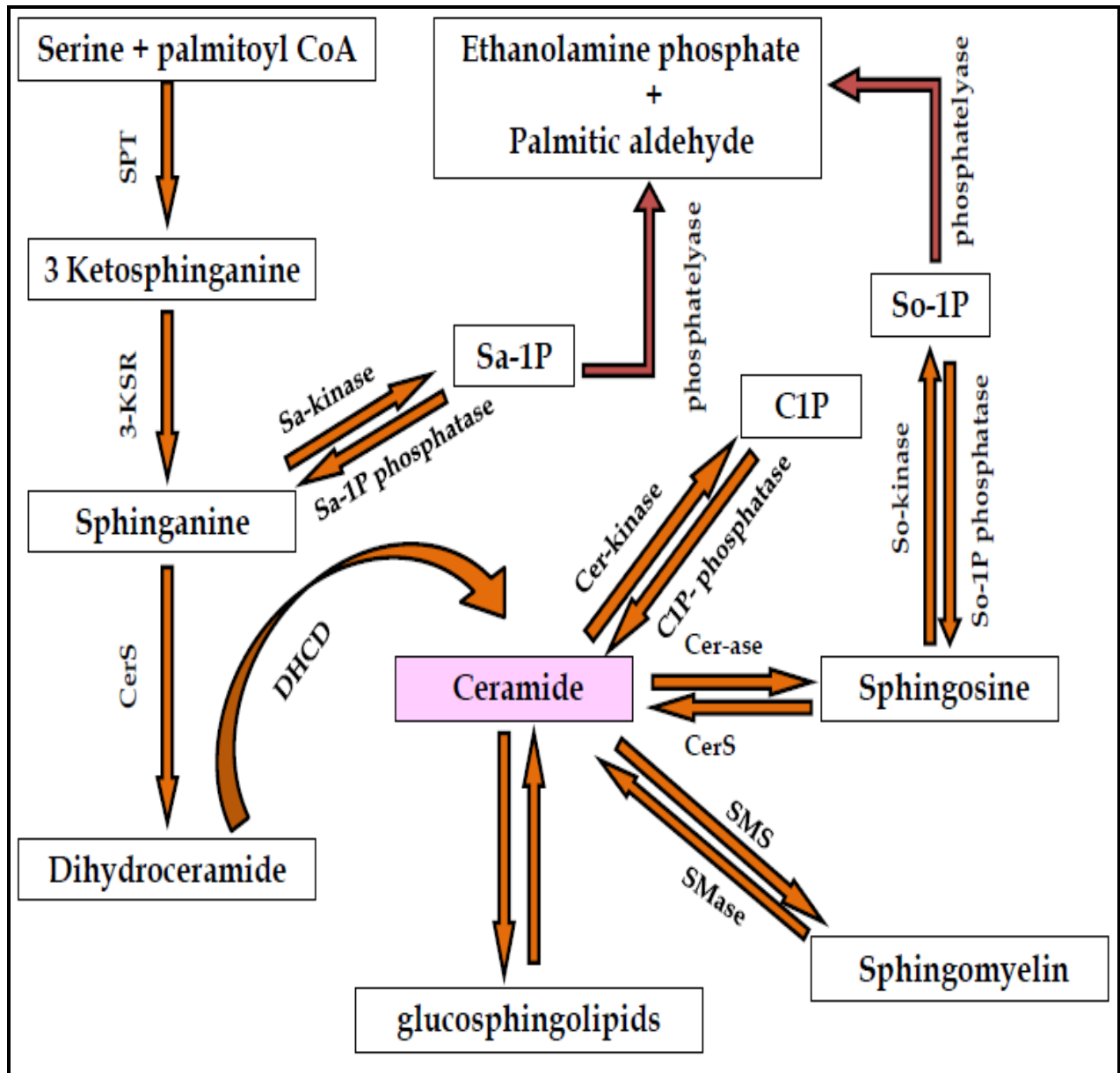


Figure 3: Diagram of sphingolipids biosynthesis [179-180-181-182]

1.3. Role of sphingolipids

Sphingolipids have important roles in cell regulation such as cell structuring, cell arrangement, cell growth, cell differentiation and death. They regulate signal transduction pathways during apoptosis, adhesion, cell proliferation, differentiation and migration and act as pro and anti-inflammatory mediators, (figure 4) [176-188-189-197]. Intracellular accumulation of free sphingolipids (Sa, So and ceramide) leads to potential inhibition of protein kinase C (PKC) [190-199-214-215-250]. This enzyme has stimulant effect on the kinas enzyme, which leads to reducing of free

sphingolipids accumulation in the cells by enhancing sphingolipids phosphorylation. Therefore, PKC enzyme is recognized as a promoting factor for cell proliferation and anti-apoptosis. Consequently, inhibition of PKC by accumulation of free sphingolipids induces cell cycle arrest and apoptosis. For that reason, accumulation of Sa, So and ceramide are deemed as cytotoxic [187-190-191-192-197-203-214-215-216].

Cytokine (TNF- α , IL-1 β and γ INF), vit-D3, endotoxin (bacterial toxin and cell membrane LPS), radiations (X-ray or UV), heat stress and oxidative stress activate sphingomyelinase, which degenerates sphingomyelin into ceramide [179-186-199-250-322]. Accordingly, those factors are reducing activity of kinase enzyme, decrease phosphorylation ability of sphingosine and ceramide, and increase conversion of sphingosine to ceramide. As a result of the above, concentration of intracellular ceramide is increased, (figure 4) [183-185-197].

Additionally, ceramide has pro-inflammatory mediator properties and enhances stimulation of IL1 and production of prostaglandins, (figure 4) [187-196]. Therefore, ceramide is implicated in the development of allergic asthmatic responses and airway inflammation, [179]. On the other hand, sphingosine and ceramide cause destabilization of the lysosomal membrane and release of lysosomal content enzymes into the cytoplasm, such as lysosomal proteases, which damages mitochondria and induces apoptosis [183-185-197-199-250].

Sufficient evidence indicates that sphingolipids phosphorylated forms (So-1P, Sa-1P and C-1P) have helpful effects on cell growth and survival by stimulating mitogen-activated protein kinase (MAPK) and extracellular regulated kinases 1-2 (ERK1-2) [193-195]. In parallel, Sa1P and So1P act as a promoting factor for cell proliferation, cell growth, anti-inflammatory and anti-apoptosis [178-179-201-235]. Therefore, the catabolism of free sphingolipids (phosphorylation) is considered a cell protective mechanism against accumulation of free sphingolipids.

However, when inflammation becomes severe or out of control, the pro-inflammatory metabolites should be blocked or at least reduced to protect the body organs from major inflammatory damage [179]. So-1P regulates the inflammatory response via its immunosuppression effect by retaining T lymphocytes in the thymus and lymph nodes [201]. It also regulates lymphocyte traffic from blood to tissues. For

example, low doses of So-1P increase lymphocyte movement from the blood into the lymphoid organs, while higher doses of So-1P inhibit this movement [202].

Ceramide one phosphate (C1P) is one of the pro-inflammatory mediators, it enhances Archidonic acid (AA) release and prostaglandin synthesis (PG2 α), (figure 5) [200]. The key element in the biosynthesis of eicosanoids is Archidonic acid (Archidonic acid is metabolized in two ways: via cyclooxygenase pathway producing prostaglandins (PG) and thromboxanes (TX), or via lipoxygenase pathway generating leukotrienes (LT)) [179-187-234]. From other side, sphingomyelinase enhances stimulation of IL1- β and production of prostaglandins by increasing ceramide production. Concurrently, inflammatory mediator IL1- β increases intracellular generation of C1P by stimulating ceramide kinase [179]. Thus, C1P acts as an anti-inflammation agent via its potential inhibition effect on sphingomyelinase (SMase), which in turn leads to a decreased generation of ceramide and pro-inflammatory mediators (IL1- β and prostaglandins). Thus, inflammation is minimized, (figure 4) [187-196].

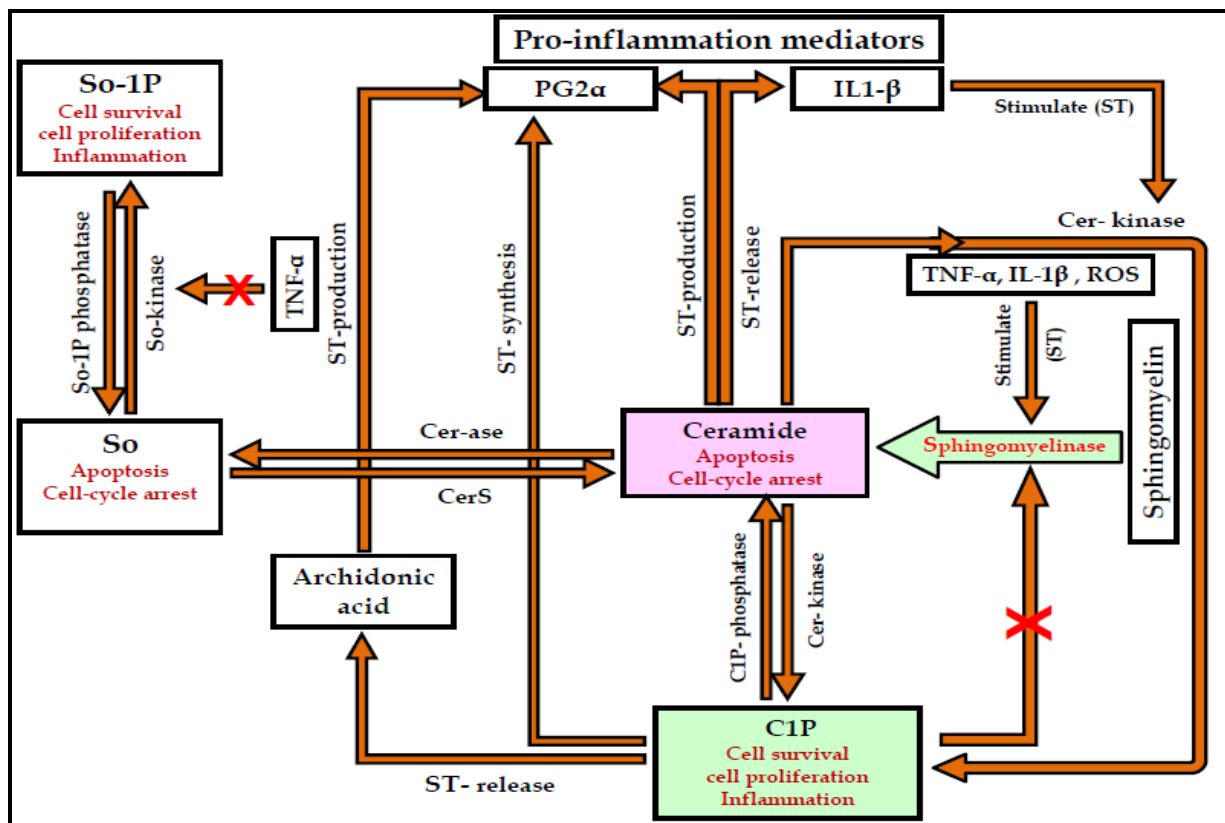


Figure 4: Role of sphingolipids in inflammatory responses [179-187-196]

1.4. Impacts of fumonisin on sphingolipids metabolism

Fumonisin toxicity has been observed in different animal species [25]. This toxicity could be explained by the ability of fumonisin to interfere with sphingolipids biosynthesis by blocking ceramide synthase enzyme (figure 5). The above is a result of fumonisin being structurally similar to sphinganine (Sa) and sphingosine (So) (figure 5) [25-205]. The disturbance of sphingolipids metabolism by fumonisin leads to increased intracellular sphinganine and sphingosine, which are known to be cytotoxic, (figure 5) [187-190-191-192-197-214-215].

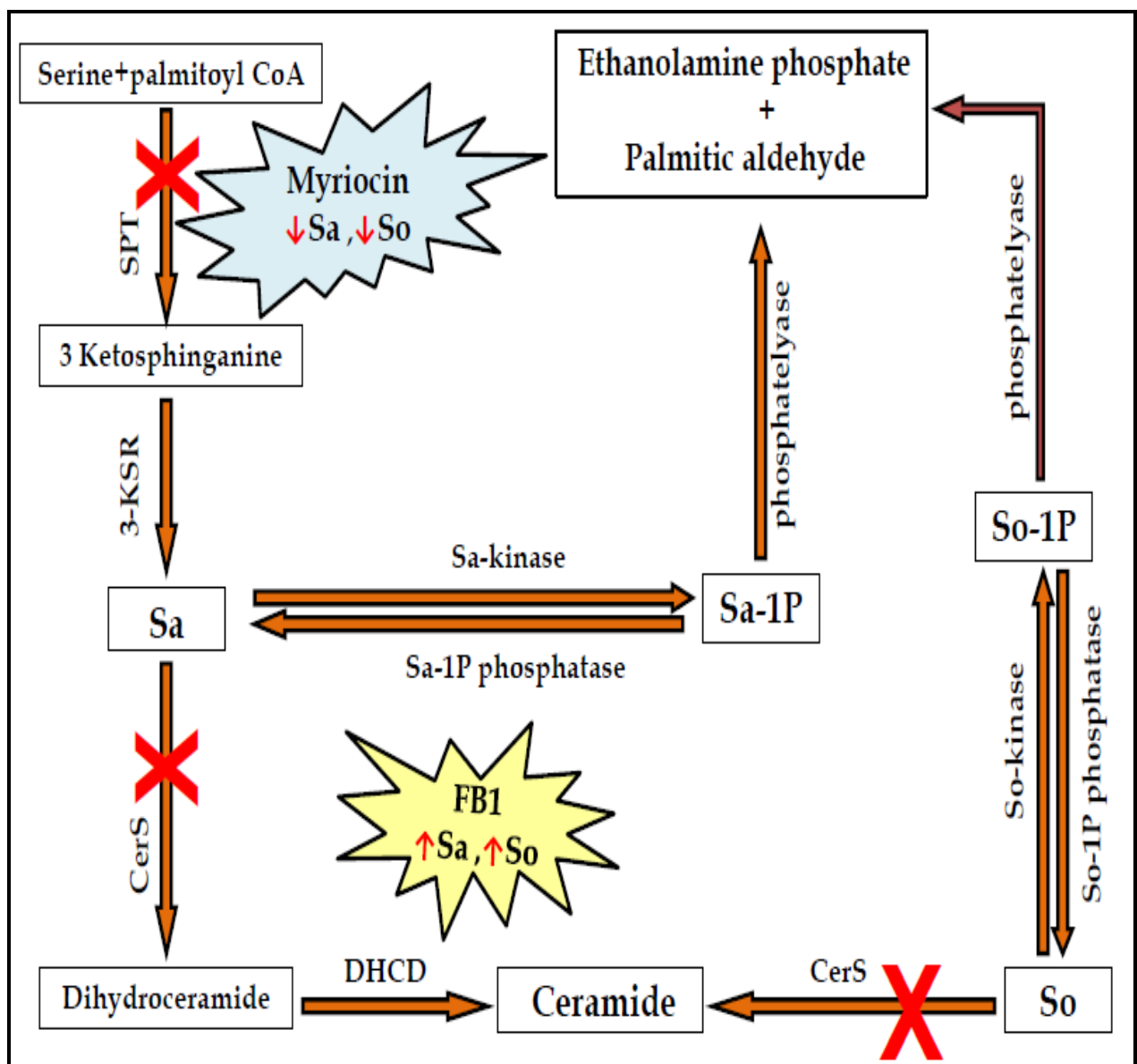


Figure 5: Impact of fumonisin on sphingolipids metabolism [87-192]

However, different studies have suggested that the hypothesis of sphingolipids metabolism disturbance by fumonisin is unable to explain the hepatotoxicity induced by a mixture of myriocin or silymarin with fumonisin.

Myriocin is potential inhibitor to serine palmitoyl transferase, which in turn prevents synthesis of sphinganine and sphingosine, (figure 5). Elevations of hepatic toxicity enzymes (ALT and AST) and sphinganine were detected in mice treated only with fumonisin, when compared to a control group. Whereas elevations of ALT and AST without elevation of sphinganine were observed in mice injected with myriocin plus fumonisin, when compared to the control group. Those results are an indication of the ability of myriocin to prevent fumonisin caused accumulation of free sphingolipids, while it is unable to prevent the fumonisin toxicity effect, (figure 5) [87].

Silymarin is known as a hepatic protector and antioxidant, because it has strong reduction effect on the production and release of cytokines (TNF- α , γ INF, and IL-2) and ROS from kuffer cells [65-184]. Elevations of Sa and So in mice group treated with silymarin plus fumonisin were more pronounced than in the group treated with fumonisin alone. While the elevations of hepatic toxicity enzymes (ALT and AST) were less pronounced in the group treated with silymarin plus fumonisin in comparison to the group treated with fumonisin alone. The above illustrates that a combination of fumonisin with silymarin leads to an increase in free sphingolipids (Sa and So) accumulations, and a decrease in hepatotoxicity parameters [236].

Consequently, fumonisin hepatotoxicity does not depend on the accumulation of free sphingolipids inside the hepatocytes alone, as there are other fumonisin mechanisms of action to induce hepatic toxicity in existence.

2. Macrophage cells

2.1. Historical background of macrophage

Monocytes are a type of white blood cell which differentiates to macrophages when they enter damaged tissue through the endothelium of a blood vessel. Macrophages have different names depending on their location: in the connective tissue, liver, spleen, bone and neural tissue they are called Histiocytes, Kupffer cells, sinusoidal lining cells, Osteoclasts and microglia, respectively. Monocytes are attracted to a damaged site by stimulant chemical substances, including damaged cells, pathogens and cytokines, released by macrophages already at the site. [172]. Macrophages are active cells that can detect and kill extracellular micro-organisms. They perform an immunoregulatory function by secreting pro-inflammatory cytokines [IL-1 β , IL-2, IL-6, IL-8, IL-12, tumour necrosis factor- α (TNF α), and transforming growth factor- β (TGF- β)], and anti-inflammatory cytokines (IL-4 and IL-10) [234-237-238-239]. Both IL-1 β and TNF α attract neutrophils to an inflamed site, while IL-8 is a potent chemoattractant of both lymphocytes and neutrophils to the inflamed site [239]. In addition, TNF α and IL-12 stimulate T lymphocytes and natural killer cells in the liver to produce IFN γ [238]. Macrophages eliminate micro-organisms or xenobiotic by producing low molecular weight metabolite molecules which cause significant local and systemic biological changes in order to increase foreign body recognition. Those metabolite molecules are reactive oxygen species (ROS), reactive nitrogen intermediates (RNI), and the eicosanoids (prostaglandins (PG), thromboxanes (TX), and leukotrienes (LT)) [234].

2.2. Impact of fumonisin on Kupffer cells

Kupffer cells are implicated in hepatic injury induced by various hepatotoxicants compounds, such as carbon tetrachloride, acetaminophen, ethanol and cadmium [238]. It is believed that the scavenger function of Kupffer cells which removes chemical waste products induces liver damage, by producing superoxide anions and cytokines [238]. Oxidative stress and TNF α destabilize lysosomal membrane which in turn releases lysosomal content, such as lysosomal proteases, into the cytoplasm thus damaging mitochondria and inducing apoptosis [199].

Kupffer cells are the main source of superoxide anions, hydrogen peroxide, nitric oxide (NO), nitric oxide synthase (NOS) and cytokines as TNF α , IL-1 β , IL-6, and IL-12 [238]. One of the effective ways of FB1 to induce hepatic toxicity is through disturbance of the balanced secretion of pro-inflammatory and anti-inflammatory cytokines by Kupffer cells [238]. TNF α is an important mediator of FB1 hepatotoxicity in mice [238-240-241]. FB1 treatment causes an increased expression of gene cytokines in mouse liver, such as tumour necrosis factor (TNF α), interleukin (IL-1 α , IL-1 β , IL-12), and interferon (IFN γ), with the most rapid and highest augmentations observed in TNF α and IFN γ after 4 and 8 hours of treatment, respectively [240-241]. Moreover, FB1 hepatotoxicity is reduced in mice lacking either TNF α receptor (TNFR1 or TNFR 2) [242-243].

The implication of Kupffer cells in fumonisin hepatotoxicity was checked by exposing groups of mice to fumonisin alone or fumonisin plus gadolinium. Gadolinium chloride is a selective Kupffer cell toxicant that completely eliminates Kupffer cells from the liver. Significant elevations in hepatotoxicity enzymes (ALT and AST) were obtained in the group treated with FB1 alone, compared to the control and mixed treatment groups. Meanwhile, a significant diminishing in hepatotoxicity enzymes (ALT and AST) was observed in the mixed treatment group in comparison to the group treated with FB1 alone [238]. Therefore, the implication of Kupffer cells in fumonisin hepatotoxicity was proven by the ability of gadolinium to reduce fumonisin hepatotoxicity by completely eliminating Kupffer cells in the liver.

3. Conclusion

Exposure to fumonisin leads to an increase in sphinganine and sphingosine in the tissues and blood of all tested animal species. This accumulation of free sphingolipids could explain one part of fumonisin toxicity pathway. On the other hand, a significant decrease of free sphingolipids (Sa and So) without significant decrease in hepatotoxicity was observed in mice treated with fumonisin plus myriocin when compared to a group treated with fumonisin alone. By contrast, a significant increase of free sphingolipids (Sa and So) without a significant increase in hepatotoxicity was observed in mice treated with fumonisin plus silymarin when compared to the group treated with fumonisin alone. Therefore, the hypothesis that fumonisin causes hepatotoxicity through its sphingolipids metabolism disturbance, is unable to explain the hepatotoxicity induced by the mixture of myriocin or silymarin with fumonisin. Subsequently, fumonisin hepatotoxicity is not only dependent on the accumulation of free sphingolipids inside the hepatocytes, but it also appears that another fumonisin mechanism of inducing hepatic toxicity exists.

Other hypotheses of the fumonisin toxicity pathway are: i) Ability of fumonisin to disturb the free sphingolipids phosphorylation mechanism. ii) Ability of fumonisin to disturb the secretion of pro-inflammatory and anti-inflammatory cytokines, and secretion of ROS by Kupffer cells

VI. Fumonisin biomarkers

Fumonisin is distributed worldwide, and they frequently contaminate maize at high concentrations. Taking into account that maize is the most important cereal grain in animal diets, and it is the third most important cereal grain for human consumption. Therefore, many efforts have been made to find biomarker(s) of FB1 exposure in animal species, as well as in humans.

Fumonisin has been reported to induce hepatic and kidney toxicity in all the animal species (chapter III). Hepatic toxicity goes with alteration of biochemistry that is not specific of FBs exposure. Because FB1 block ceramide synthase (chapter V), alteration of sphingolipids was also reported in all species. So, biochemistry and sphinganine levels in tissue and serum have been proposed as biomarkers of fumonisins. After a general presentation of what is a biomarker, this chapter will present the biomarkers used during FB1 exposure. A specific focus will be done on the smallest FBs exposure that could be detected by using each kind of biomarker, in terms of dose and duration of exposure. Moreover, the consequences of these effects in terms of toxicity will be reported. Indeed, although alterations of sphingolipids metabolism occur during FBs exposure, the link between these alterations and cell damage is complex.

1. General aspect of biomarkers

1.1. Identification and classification of biomarkers parameters

Biomarkers of xenobiotic exposure are divided in: direct biomarker (biomarkers of exposure) and indirect biomarker (biomarker of effect). A biomarker of exposure is a quantification of the original compound, or its metabolites, in a body compartment or fluid, which is dependent on its pharmacokinetics [263]. A biomarker of effect is the presence of certain biological response following exposure to a xenobiotic [263]. All biomarkers are evaluated by two aspects: sensitivity and specificity [262]. Sensitivity is related to detection of the biomarker before the toxic effects or a clinical sign of toxicity appear [261]. Specificity means that the biomarker is specific for an agent, and not to other unrelated agents [261].

1.2. Biomarker of FB1 exposure (direct biomarker)

Direct detection of FB1 in serum or urine is difficult, because it depends on many factors as follows:

- Scenario of toxin exposure: humans have interval exposure to FB1, because their food does not mainly include corn or corn products. Whereas animals have regular exposure to FB1 due to their feed mainly consisting of corn or corn products.
- Bioavailability: lower bioavailability of FB1 compared to other mycotoxins leads to a decrease in the possibility of direct detection of FB1 in blood and urine. For example: The bioavailability of aflatoxin and ochratoxin are: 50%, and less than 40% of administered dose, respectively. Whereas, the bioavailability of FB1 is less than 5% of administered dose in different animal species [25-265-288].
- Elimination half-life: sampling time after exposure to FB1 is one of the points that play an important role in direct detection of FB1 in serum or urine. For example, elimination half-life of aflatoxin and ochratoxin are approximately 92 hr, and 320 hr, respectively. Whereas elimination half-life of FB1 is approximately 3 hr, depending on animal species [25-265-266-288].
- Sensitivity of the measuring apparatus: TLC (thin layer chromatography) is low sensitive apparatus to FB1 detection, and it is unable to detect FB1 at low concentrations as expected in blood or urine. Because it has high limit of detection (1000 ng FB1/g) [18]. By contrast, HPLC or HPLC-MS are high sensitive apparatus to FB1 detection, because they have low limit of detection (13 and 5 ng FB1/g or ml [23-24-284].

1.3. Biomarker of FB1 effects (indirect biomarker)

It is divided into specific biomarker (sphingolipids parameters) and non specific biomarker (hepatotoxicity parameters).

1.3.1. Specific biomarker (Sa, So and Sa:So ratio)

Specificity of free sphingolipids as a biomarker to exposure of FB1 was concluded from the following several points:

- Disturbance of sphingolipids metabolism by fumonisin was recorded in all observed animal species [25-81-150]. Free sphingolipids are widely distributed in the body, which is present in all tissue cells, and disturbance of sphingolipids metabolism by FB1 leads to leakage of intercellular free sphingolipids to extracellular, then to blood and urine [25-81-150-173-174-175].
- Elevation of Sa and Sa:So ratios in different body compartments (tissue, blood, and urine) are observed within few hours post-exposure to a low dose of FB1, and remain elevated for several days or weeks, depending on animal species [63-171-274-285].
- Very few types of molecules (such as myriocin and silymarin) can interfere with FB1 effects on the sphingolipids metabolism. Myriocin, having an antagonistic effect on FB1, leads to a decreased production of free sphingolipids (Sa and So). Whereas, silymarin, having a synergic effect on FB1, leads to an increased production of sphingolipids [87-236].

1.3.2. Non specific biomarker (hepatotoxicity parameters)

There are some parameters which are used to find out the extent of FB1 effects on the body organs, such as total protein (TP), total cholesterol (CHOL), alanine transaminase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), gammaglutamyl tranferase (GGT), creatinine kinase (CK), and alkaline phosphatase (ALP). However, these parameters are considered as non specific and non sensitive biomarkers to FB1 exposure due to the following reasons:

- Several agents can increase those parameters, such as infectious agents (viral and parasitic), drugs (amoxicillin/clavulanic acid, aspirin, paracetamol, etc), or mycotoxins (aflatoxin B1, deoxynivalenol and zearalenone) [245-300-301].

Moreover, those parameters are present in several tissues, and any disturbance of those tissues leads to secreting them in the body fluid [245-246-251].

- Those parameters are elevated in the serum after several days post-exposure to a high dose of FB1, depending on sex and species [63-146-149-150-155-171-264-275].

1.3.2.1. Total Protein (TP)

Serum protein includes albumin, globulins, fibrinogen and lipoproteins [219-249]. All the serum proteins are synthesized in the liver except gamma globulins, which are synthesized in the lymph nodes [220]. The main functions of a serum protein are: control of extracellular fluid distribution, control of infections by producing immunoglobulin (antibodies), transport function (albumin and specific binding proteins), control of clotting process (fibrinogen) and essential source of tissues nutrition [219]. Thus, the total protein assay is used to assess hydration status, nutritional status, liver function (capacity of liver biosynthesis), and kidney function [221].

Hypoproteinemia comes from exogenous causes (lack of protein in diet, intestinal parasites or malabsorption syndrome) and endogenous causes, such as liver disease that lead to decreased serum protein synthesis (albumin), and kidney disease which leads to escape of proteins in the urine (albuminuria) [219]. Whereas, hyperproteinemia is caused by dehydration (lack of water intake or severe diarrhea), chronic liver disease and chronic infection [177-219].

In avian, the causes of hypoproteinemia and hyperproteinemia are similar to those in mammals. Hyperproteinemia is commonly observed in acute inflammatory processes, and late stages of chronic liver disease [164-246-294-295].

1.3.2.2. Total Cholesterol (TC)

Cholesterol is an essential molecule for cell membranes, and it is the originator of corticosteroid hormones, sex hormones, bile acids, and vitamin D. All tissues containing nucleated cells are capable of cholesterol synthesis, which occurs in the endoplasmic reticulum and the cytosol. The liver and intestines produce about 20-

25% of total daily cholesterol [223]. Cholesterol is present in tissues and in serum either as a free cholesterol form or combined with a long-chain fatty acid as a cholesteryl ester. [222-223]. There are four major lipoproteins responsible for cholesterol and fatty acid transportation inside the body (Chylomicrons, LDL, VLDL and HDL). Chylomicrons are vehicles transporting dietary absorbed fats (fatty acids, cholesterol and cholesteryl ester) from the intestine into the lymph vessels, then directly to the blood circulatory system, without passing through the liver (liver bypass) [223-303]. Low-density lipoprotein (LDL) and very low density lipoproteins (VLDL) are the vehicles of uptake of fatty acids and cholesterol from the blood into many tissues. Cholesterol is removed from the tissues by high-density lipoprotein (HDL) and transported to the liver, where it is eliminated from the body either unchanged or after conversion to bile acids. Cholesterol is a major constituent of gallstones, and is an essential factor of atherosclerosis genesis [223].

Avian cholesterol consists of free cholesterol and cholesterol esters which are measured together as total cholesterol in mammals. It is an important compound forming hormones, bile acid and salts, as well as the plasma membrane of cells. Avian cholesterol is produced by all nucleated cells, mainly in the liver and intestine as with mammals [85-246-294-295]. Avian cholesterol is transported inside the body by lipoproteins (VLDL, LDL, and HDL) as in mammals [305-307]. However, the absorption pathway of dietary fats (fatty acids and cholesterol) is totally different from mammals. The absorbed fats are transported by lipoprotein called portomicrons from the intestinal tract into the portal vein. Then they go directly to the liver, where they are metabolized and transported to the blood circulatory system and bile duct [302]. The accumulation of fats in liver (hepatic steatosis) is a normal physiological condition in migrating birds and ducks [158-302-311], while it is a pathological condition (steatohepatitis) in non-migrating birds and mammals [223-302-306]. Hypercholesterolemia was reported during acute hepatic steatosis, bile duct obstruction, liver disease, starvation, high fat diets and obese birds. Whereas, decrease of serum cholesterol level is associated with aflatoxicosis, reduction fat in diet and E Coli endotoxemia [85-246-294-295].

1.3.2.3. Aminotransferases

Aminotransferases include alanine transaminase (ALT) and aspartate transaminase (AST). These enzymes are utilized frequently as specific indicators for liver necrosis [245].

Alanine transaminase (ALT) is found predominantly in the liver, and is located only in the cytoplasm of hepatocytes. Elevation of ALT in the serum is specific and sensitive indicator for hepatitis, which leads to increased hepatocellular membrane permeability [245-308]. AST is present in both the cytoplasm (20%) and mitochondria (80%) of hepatocytes and many other cells (heart, skeletal muscles, kidneys, brain, pancreas and red blood cells) [245-308]. Any alteration of cell membrane permeability leads to slight elevation of AST in the serum as in the case of hepatitis. Whereas, severe cell damage leads to high elevation of AST in the serum, due to release of the enzyme from cytoplasm and mitochondria as hepatic necrosis [308]. AST is also an indicator of myocardial infarction, acute pancreatitis, acute hemolytic anemia, acute renal disease, musculoskeletal diseases, and trauma [245]. Therefore, ALT is a more specific and sensitive indicator to hepatocyte injury than AST [245]. Elevation of serum ALT and AST levels are indicated to viral, drug, toxin induced hepatitis or necrosis, and acute biliary tract obstructions. On the other hand, normal elevation of serum ALT is observed with physical exercise or in dietary choline deficiency. Moreover, ALT normally fluctuates over the course of time [251]. Declining of ALT and AST are indication of either hepatocyte recovery, or hepatocyte being unable to further release these enzymes as in the case of hepatic failure [245].

In avian, Alanine transaminase (ALT) and Aspartate transaminase (AST) are not specific indicators of hepatic injury, in contrast to mammals, because they are produced by muscle atrophy. Therefore, more specific tests of muscle atrophy, such as creatinine kinase (CK) are needed to confirm the elevation cause of those enzymes (ALT and AST) in avian [246-247-294-295].

1.3.2.4. Lactate dehydrogenase (LDH or LD)

LDH is an enzyme located in the cell mitochondria of most body tissues. Therefore, complete cell disruption is necessary before it is released in large quantities. There are five sub-classes of LDH, which are distributed in specific tissues

as follows: LDH-1 and LDH-2 are found predominantly in heart, kidneys and erythrocytes. LDH-3 is most found in the lungs, whereas LDH-4 is present in kidneys, placenta and pancreas. LDH-5 is found in the liver and striated muscles. The elevation one of LDH sub-classes in serum is indicator to disturbance in specific tissues [244-251]. Elevation of LHD in the serum occurs during cell necrosis or hemolysis conditions, such as anemia or incorrect handling of blood samples in vitro [173-251-308].

In avian, LDH is distributed in a wide variety of tissues as it is in mammals. However, there is no information about sub-classes of LDH, in contrast to mammals. Therefore, LDH elevation in avian serum is not specific to any organs. In addition, LDH is an unstable enzyme that rises and falls more quickly in serum than AST. Therefore, LDH is considered a poor indicator for liver damage in avian. Regardless of that, the common cases which lead to increased LDH in serum of avian are liver necrosis, muscular damage, and blood hemolytic conditions. In the case of body weight decrease, differentiated test of muscle atrophy, such as creatine kinase (CK) is necessary [246-247-294-295-308].

1.3.2.5. Gamma-Glutamyl Transferase (GGT)

GGT is present in cell cytoplasm and also bound to cell membranes of many tissues such as, kidneys and pancreas, with more pronounciation in the liver. Therefore, it is considered specific biomarker for liver health status. GGT plays an important role in the transfer of amino acids across the cellular membrane, leukotrienes metabolism and intracellular regulation of reactive oxygen species by synthesis and degradation of glutathione (potential antioxidant) [248-251-308]. Elevation of serum GGT activity is a biomarker for acute viral hepatitis, bile duct obstruction (cholestasis), pancreatitis or drug administration (paracetamol and phenobarbitone) [245-251-308]. Slight elevation of GGT is observed in cardiovascular and circulatory diseases, such as atherosclerotic plaques [308].

In avian, GGT enzyme is more present in the biliary and renal tubular epithelium. In addition, elevation of this enzyme in the serum is commonly due to a biliary origin. Therefore, it is the most specific indicator of cholestatic liver disease.

However, it is a lower sensitivity indicator of liver disease when compared to AST [246-295].

1.3.2.6. Alkaline phosphatase (ALP)

ALP is present in the plasma membrane of cells in all body tissues, with high quantities in intestines, kidney, bone, bile, liver, placenta and the lactating breast [245-251-309]. ALP is normally elevated in the serum of growing children and women in the third trimester of pregnancy. ALP is used as a biomarker for some liver diseases, such as hepatitis and biliary obstruction. ALP is less specific biomarker than GGT for hepatic and biliary injury, because ALP is released in some cases, such as bone tumor disease (Paget's disease), osteomalacia, rickets and hyperparathyroidism [245-251].

In avian, ALP is mainly activated in the duodenum and kidneys, with low activity in the liver. Concurrently, other avian organs show no ALP activity in its tissues, in contrast to mammals. High elevation of ALP in serum is an indicator to enteritis. While, slight elevation of ALP is most common in hepatocellular irritation, not damage. Therefore, ALP is not specific or sensitive to hepatic damage in contrast to mammals [246-295].

2. Uses of biomarkers in FB1 exposure

This part will present the biomarkers of FB1 exposure in laboratory animals and farm animals (high and low sensitivity animals).

2.1. Biomarker of FB1 exposure in rodents

In male Sprague-Dawley rats, the dose required to increase Sa:So ratio in kidney and urine after 4 weeks was lower (15 mg FB1/kg of feed) than the dose required to increase Sa:So ratio in liver and serum and hepatotoxicity enzymes (150 mg FB1/kg of feed) [264]. In male-F344 rats, 5 mg FB1/kg of diet was able to elevate Sa:So ratio in kidneys and urine after 6 and 10 weeks post-feeding, respectively. Whereas, 150 mg FB1/kg of diet for 2 years was unable to elevate Sa:So ratio in liver or hepatotoxicity enzymes, such as TP, ALT, GGT, and ALP [264]. In female-F344 rats, Sa:So ratio in kidney was increased by half a dose (50 mg FB1/kg of feed)

necessary to increase Sa:So in liver (100 mg FB1/kg of feed) after 6 weeks of exposure. Moreover, hepatotoxicity enzymes were not elevated in female-F344 rats fed 150 mg FB1/kg of diet for 2 years, (table 16)[264].

Table 16: Effects of FB1 exposure on biomarkers (Sa:So and hepatic enzymes) in rodents

Species	Sa:So Kidney/Urine	Sa:So Liver/Serum	Biochemistry*	Effect	Ref
Rat SD Male	15 mg/kg/4 wks	150 mg/kg/4 wks	150 mg/kg/4 wks	LOEL	[264]
		50 mg/kg/4 wks	50 mg/kg/4 wks	NOEL	
Rat F344 Male	5 mg/kg/10 wks			LOEL	[264]
	99 mg/kg/1 wks		484 mg/kg/1 wks	NOEL	
			234 mg/kg/4 wks		
		150 mg/kg/2 years	150 mg/kg/2 years		
Rat F344 Female	50 mg/kg/6 wks	100 mg/kg/6 wks		LOEL	[264]
	99 mg/kg/1 wks		484 mg/kg/1 wks	NOEL	
			234 mg/kg/4 wks		
	15 mg/kg/2 years	50 mg/kg/2 years	150 mg/kg/2 years		
Mice B6C3F1 Male female	234 mg/kg/1 wks		484 mg/kg/1 wks	LOEL	[264]
	163 mg/kg/4 wks		234 mg/kg/4 wks	NOEL	
Mice B6C3F1 Female		10 mg/kg/4 wks	50 mg/kg/4 wks	LOEL	[275]
			10 mg/kg/4 wks	NOEL	

Dose: mg FB1/kg of feed; Sa :So : sphinganine/sphingosine ratio

*LOEL (lowest-observed-effect-level) or NOEL (No -observed-effect-level) observed on alanine aminotransferase, aspartate transaminase, alkaline phosphatase and cholesterol

In female and male F344 rats, the dose required to elevate Sa:So ratio in urine (99 mg FB1/kg of diet) was lower than the dose required to elevate Sa:So ratio in the urine of male and female B6C3F1mice (234 mg FB1/kg of diet), after one week of feeding [264]. Surprisingly, urinary Sa:So ratio in rats and mice fed a diet at concentration 99 and 234 mg FB1/kg, respectively, were significantly elevated between the 7th and 14th days in rats and only at the 7th day in mice, after that they declined to no significant levels at the 28th day, (table 16) [264].

In female B6C3F1 mice, the dose required to elevate serum Sa:So ratio was lower (10 mg FB1/kg of diet) than the dose required to increase hepatotoxicity parameters, such as cholesterol and ALP (50 mg FB1/kg of diet), for 28 days, (table 16) [275].

In male F344 rats, serum and urinary Sa:So ratio were quickly elevated after 12 hours, and remained so far 8 days before returning to control level at day 10 post-dosing with a single oral dose of 10 mg FB1/kg b.w. (table 16) [285].

It is apparent from those studies that sensitivity of rodents to FB1 exposure was different according to exposure dose, animal strains, animal sex, and animal organs. On the other side, the required dose of FB1 to elevate hepatotoxicity parameters was higher than the required dose to elevate Sa and Sa:So in fluid and tissues. Also, the required dose of FB1 to increase Sa and Sa:So in the serum was higher than the one for tissues. Moreover, FB1 effects on sphingolipids metabolism and hepatotoxicity enzymes were more pronounced with short term exposure than with long term exposure and higher than NOAEL based on kidney toxicity during chronic exposure in rat used for the determination of the PMTDI (page 34).

2.2. Biomarker of FB1 exposure in farm animals

2.2.1. Horses and swine

Sphinganine and sphinganine to sphingosine ratio in serum rapidly elevated two days post-feeding ponies with contaminated diets at level 44 mg/kg of feed. Hepatotoxicity parameters (ALT and AST) and ELEM signs appeared later ten days post-feeding [252]. In another study, sphinganine and Sa:So ratio of serum increased at equivalent oral doses of 0.2 mg/kg b.w./day for 28 days. Hepatotoxicity and ELEM were observed at equivalent oral doses ≥ 1 mg/kg b.w. /day for 28 days [150-191-193].

Elevation of Sa:So and hepatotoxicity parameters in serum and ELEM signs did not appear in horses fed 15 mg FB1/kg of diets during 150 days [290]. A similar result was found with a pony fed on a diet containing maize with 15 mg FB1/kg during 130 days [252].

In piglets, Sa:So ratio in serum and urine were rapidly elevated after a single oral dose of 5 mg FB1/kg b.w. (6 and 12 hr post-dosing, respectively), whereas, toxicity signs and hepatotoxicity parameters was detected later (24 and 96 hr post-dosing, respectively) [63].

Obviously, that disturbance of sphingolipids occurred rapidly before the appearance toxicity signs and elevations of hepatotoxicity enzymes. Besides that, the required dose to increase Sa and Sa:So ratio was lower than the required toxicity dose.

2.2.2. Ruminants

The information concerning biomarkers to FB1 exposure in ruminants is poorly documented.

In calves, hepatotoxicity parameters, such as AST, GGT, LDH, and cholesterol increased 10 days after consumption of 148 mg FBs/kg of feed [133].

In Holstein steers, AST and GGT were elevated after 253 days of feeding on 94 mg FB1/kg diet, other hepatotoxicity parameters, such as total protein, cholesterol and LDH were not affected [138].

Meanwhile, in angora goats, cholesterol, AST, GGT, and LDH were elevated after 112 days of feeding on 94 mg FB1/kg diet [142].

Unfortunately, the effect of FB1 on sphingolipids metabolism was not studied in those experiments.

2.2.3. Poultry

The fixed standards of specific and non specific biomarker of fumonisin exposure were not available in poultry, because they were dependent on many factors such as species, sex, age, body temperature, feed quality, and hydration states [219-248-249].

2.2.3.1. Layer hens and broiler

In laying hens, decrease in serum total protein and increase in AST activity were observed in groups fed 200 mg FB1/kg, for 112 days. Concurrently, GGT, ALP, or LDH were not affected in groups fed 200 mg FB1/kg of feed for 420 days [155]. In

one-day-broilers which consumed contaminated diet for 21 days, AST and cholesterol increased at 300 mg FB1/kg of feed. But ALP, LDH and total protein were not affected at 400 mg FB1/kg of feed, (table 17) [148].

Table 17: Effects of FB1 exposure on biomarkers (Sa:So and hepatic enzymes) in chickens

Species	Effet	Sa :So ratio	Biochemistry	Ref
Laying hens White Leghorn	LOEL		200 mg/kg/112d: TP, AST	[155]
	NOEL		100 mg/kg/420d: TP, AST 200 mg/kg/420d: CHOL, ALT, GGT, LDH, ALP	
Broiler	LOEL		100 mg/kg/21d: AST 200 mg/kg/21d: CHOL	[148]
	NOEL		400 mg/kg/21d: TP, LDH, ALP	
Broiler Arbor Acres	LOEL	20 mg/kg/21d liver 80 mg/kg/day serum	80 mg/kg/21d: AST	[146]
	NOEL		80 mg/kg/21d: CHOL, LDH, ALP	
Broiler Cornish cross	LOEL	25 mg/kg/42d liver		[153]
	NOEL		50 mg/kg/42d: TP, CHOL, AST, GGT, LDH, ALP	

Dose: mg FB1/kg of feed; LOEL: lowest-observed-effect-level; NOEL: No -observed-effect-level; NC: not conducted; Sa :So : sphinganine/sphingosine ratio; TP: total protein ; ALT: alanine aminotransferase; AST : aspartate transaminase ; GGT : Gamma-glutamyltransferase ; LDH : Lactate dehydrogenase ; ALP : Alkaline phosphatase; CHOL, cholesterol

Unfortunately, Sphinganine and sphinganine to sphingosine ratio were not tested in both experiments.

In one-day-broiler chickens fed a contaminated feed for 21 day, Sa:So ratio in liver increased at the group fed with 20 mg FB1/kg feed, and Sa:So ratio of serum and AST increased at the group fed with 80 mg FB1/kg feed. Other hepatic parameters, such as cholesterol, ALP and LDH were not affected by the 80 mg FB1/kg feed (table 17) [146].

In one-week old broilers, 25 mg FB1/kg of diet for 42 days was sufficient to induce a significant elevation of Sa and Sa:So ratio in the liver. By contrast, 50 mg FB1/kg of feed for 42 days was unable to elevate serum hepatotoxicity parameters, (table 17) [153].

2.2.3.2. Turkeys

In one-day old turkeys, Serum Sa:So ratio was increased by feeding them with 75 mg FB1/kg of feed for 21 days. Unfortunately, hepatotoxicity parameters were not measured in this study (table 18) [154].

Table 18: Effects of FB1 exposure on biomarkers (Sa:So and hepatic enzymes) in turkeys

Species	Effet	Sa :So ratio	Biochemistry	Ref
	LOEL	75 mg/kg/21d serum		[154]
	NOEL			
	LOEL		100 mg/kg/21d: CHOL, AST, ALP	[310]
	NOEL		200 mg/kg/21d: TP, GGT	
	LOEL	25 mg/kg/21d-liver	325 mg/kg/21d: ALT, GGT	[149]
	NOEL		250 mg/kg/21d: ALT, GGT	
BUT 9	LOEL	5 mg/kg/49d liver, kidneys 20 mg/kg/7d liver, kidneys		[156]
	NOEL	20 mg/kg/63d serum	20 mg/kg/63d: TP, CHOL, AST, LDH, ALP	
Nicholas	LOEL	25 mg/kg/84d - liver		[153]
	NOEL	50 mg/kg/84d- serum	50 mg/kg/84d: TP, CHOL, AST, GGT, ALP	

Dose: mg FB1/kg of feed; LOEL: lowest-observed-effect-level; NOEL: No -observed-effect-level; NC: not conducted; Sa :So : sphinganine/sphingosine ratio; TP: total protein ; ALT: alanine aminotransferase; AST : aspartate transaminase ; GGT : Gamma-glutamyltransferase ; LDH : Lactate dehydrogenase ; ALP : Alkaline phosphatase; CHOL, cholesterol

In one-day turkeys fed a contaminated diet for 21 days, liver Sa:So ratio increased with a dose of 25 mg FB1/kg of feed. Serum GGT and AST were elevated at a dose of 325 mg FB1/kg of feed,(table 18) [149].

In one-day old turkeys, elevation of Sa:So ratio in liver and kidneys was observed quickly at 7 days after feeding with 20 mg FB1+ FB2/kg of feed. Whereas, Sa:So in serum and hepatotoxicity parameters remained nearly constant over a period of 9 weeks. Moreover, elevation of Sa:So ratio in tissues was dose-dependent. It increased at 49, 35, and 7 days post-feeding turkeys with 5, 10 and 20 mg FB1+ FB2/kgof feed, respectively. Also, the liver and the kidneys seem to have the same sensitivity to fumonisin exposures, (table 18) [156].

In one-day old turkeys, cholesterol decreased, and AST and ALP increased after 21 days of receiving 100 mg FB1/kg of feed, whereas, protein and GGT was not affected by feeding 200 mg FB1/kg of feed during the same period, (table 18) [310].

In one-week old turkeys, Sa and Sa:So ratio in liver significantly increased at a dose of 25 mg FB1/kg of feed for 3 months. Meanwhile, hepatotoxicity parameters were not affected by a dose of 50 mg FB1/kg of feed for the same period, (table 18) [153].

2.2.3.3. Ducks

In one-day old ducks, sphinganine and Sa:So ratio in serum, liver and kidney were increased at a very low dose of 2 mg FB1/kg feed, for 7 days. Concurrently, hepatotoxicity parameters, such as cholesterol, ALT, LDH and ALP were increased at a dose of 32 mg FB1 /kg, for 7 days, and total protein was increased at a dose of 128 mg FB1/kg, for 7 days, while, AST and GGT were not affected at 128 mg /kg, during the experimental period of 77 days. The highest increase values of Sa and Sa:So were recorded in the kidneys and not in the liver. Thus, it was suggested that the kidney is the most sensitive organ to FB1 exposure. Interestingly, in this study, Sa:So ratio in serum and tissues, and hepatotoxicity enzymes declined after 7 to 21 days post-intoxication. The effects of FB1 on Sa:So ratio in serum and tissues, and hepatotoxicity parameters from 49th to 77th days were nearly constant. Thus, it appears that cumulative dose of FB1 in the tissues and the serum has a more extensive effect on the Sa:So ratio at a short period (7-21 days) compared to a longer period (42-77 days). For example, the mean Sa:So ratio increased from 0.2 to 6.7 in ducks receiving a cumulative dose of 55 mg FB1 over 7 days, although it only increased from 0.2 to 0.5 when ducks received a cumulative dose of 365 mg over 49 days, (table 19) [165-170-171].

Table 19: Effects of FB1 exposure on biomarkers (Sa:So and hepatic enzymes) in ducks

Species	Effet	Sa :So ratio	Biochemistry	Ref
Mule Male	LOEL	2 mg/kg/7d serum, liver, kidneys	128 mg/kg/7d: TP 32 mg/kg/7d: CHOL, ALT, LDH, ALP	[165-170-171]
	NOEL		128 mg/kg/77d: AST, GGT 32 mg/kg/77d: TP 8 mg/kg/77d: CHOL, ALT, LDH, ALP	
Mule Male	LOEL	60 mg/kg/6d serum*	60 mg/kg/8d*: TP, CHOL, ALT, GGT, LDH	[163-164]
	NOEL	60 mg/kg/5d serum*	60 mg/kg/7d*: TP, CHOL, ALT, GGT, LDH	
Mule Male**	LOEL	10 mg/kg/12d - liver	10 mg/kg/12d: TP, CHOL, ALT, LDH	[158]
	NOEL		20 mg/kg/12d: GGT, ALP	
Pekin Male	LOEL	100 mg/kg/21d serum	100 mg/kg/21d: GGT	[162]
	NOEL		400 mg/kg/12d: TP, CHOL, AST	

Dose: mg FB1/kg of feed; * Obtained by calculation from the dose administered by gavage

** Study conducted during force feeding; LOEL: lowest-observed-effect-level; NOEL: No - observed-effect-level; NC: not conducted; Sa :So : sphinganine/sphingosine ratio; TP: total protein ; ALT: alanine aminotransferase; AST : aspartate transaminase ; GGT : Gamma-glutamyltransferase ; LDH : Lactate dehydrogenase ; ALP : Alkaline phosphatase; CHOL, cholesterol

In 6-week old mallard ducks, Sa and Sa:So ratio in the serum were increased rapidly after daily received of 5 mg FB1/kg b.w. by oral route, for 2 and 6 days, respectively. Whereas, hepatotoxicity parameters were elevated later after 8 days post-dosing, (table 19) [163-164].

In 12-week old mallard ducks, Sa:So ratio in the liver and serum, and hepatotoxicity enzymes such as protein, cholesterol, ALT, and LDH were elevated after force-feeding 10 mg FB1/kg of feed, for 12 days. In contrast, GGT and ALP were constant after force-feeding 20 mg FB1/kg of feed, for 12 days. Interestingly, in this study force-feeding does not create any alteration in sphingolipids metabolism of the control group. By contrast, hepatotoxicity parameters were increased, (table 19) [158].

From those studies it became clear that: ducks were more sensitive to FB1 exposure compared to laying hens, broilers and turkeys. The required dose to elevate Sa and Sa:So ratio in serum and tissues was lower than the required dose to elevate hepatotoxicity enzymes. Sa and Sa:So ratio were more sensitive in the liver than in the serum. Except in ducks, 2 mg FB1/kg of feed for one week was able to increase Sa and Sa:So in the serum and tissues. In ducks, a disturbance of sphingolipids metabolism by FB1 was more pronounced in kidneys than in liver, whereas in turkeys it was quite similar in both tissues. Aspartate transaminase (AST) was more sensitive to FB1 exposure compared to other hepatotoxicity enzymes in laying hens, broilers and turkeys. By contrast, AST was not affected at a high dose (128 mg FB1/kg of feed) for a long period (77 days) compared to other hepatotoxicity enzymes in ducks. FB1 had clearly adverse effects on sphingolipids metabolism and hepatotoxicity enzymes at short exposure periods rather than long exposure periods.

2.3. Biomarkers of FB1 exposure in human

2.3.1. Biomarker of FB1 exposure (Urinary FB1)

In Mexico, significant correlation between urinary FB1 (UFB1) and maize tortilla intake was recorded during a comparative study of three different consumption regions (high, medium or low). The average of urinary FB1 levels was threefold higher in women from a high consumption region than women from a low consumption region [272].

In China, a significant correlation between urinary FB1 and dietary FB1 intake was obtained in Huaian and Fusui regions. The estimated daily FB1 exposures in those areas were greater than the Provisional Maximum Tolerable Daily Intake (PMTDI) of 2 µg/kg b.w. [273]. Also, significantly correlation between urinary FB1 and dietary FB1 intake was obtained in people from South Africa, who prepared and consumed maize porridge meals from home-grown maize. The mean probable daily intake (PDI) of FB1 was 4.84 mg/kg b.w. /day. The urinary samples were collected two times per day (12 hr post meal) [271].

Therefore, the authors suggested that urinary FB1 could be a potential biomarker for FB1 exposure. This explication was not precise because direct detection

of FB1 in urine was dependent on many factors, such as scenario of toxin exposure, bioavailability of FB1, sampling time, and sensitivity of apparatus used to detect FB1 in serum or urine (LOD), as previously explained in this chapter.

2.3.2. Biomarker of FB1 effects (sphingoid bases and their ratio)

In recent years a lot of studies have been conducted to link dietary FB1 to sphingolipids disturbances in human serum and urine.

In China, a significant correlation between urinary Sa:So ratio and dietary FB1 intake was recorded in the regions of Huaian and Fusui, where the volunteers consumed contaminated diet at a high dose of FB1 (110 µg/kg b.w. per day) for one month. Men urinary Sa:So ratio increased three times higher compared to the control group of men. While, women urinary Sa:So ratio was unchanged, (table 20) [267]. Adverse results were reported at the same regions (Huaian and Fusui) in China, which demonstrated no correlation between dietary FB1 intake and Sa:So ratio in serum and urine in both areas [273].

Table 20: Biomarkers of FB1 in human

Regions	Intake of maize	Sa:So in urine	Sa:So in serum	Ref
China (Huaian and Fusui)	110 µg FB1/kg b.w. / day, one month	Men: increased threefold Women: not affected		[273]
Southern Brazil	high	1.57		[268]
Northern Argentina	high	0.69		
Central Argentina	low	0.36		
Southern Italy	low	0.36		
South Africa Bizana	6.7 to 5.8 µg FB1/ kg b.w./ day	Low (woman > men)	High	[269-270]
South Africa Centane		High (woman > men)	Low	

Significant different elevations of urinary Sa:So ratio were recorded between two populations with high maize consumption in northern Argentina (0.69) and southern Brazil (1.57) [268]. Concurrently, similar urinary Sa:So ratio was obtained between two populations with low or no maize consumption in central Argentina

and southern Italy (0.36). The probable daily intake of FB1 (PDI) in high maize consumption areas was similar. The two populations of high maize consumption were considered treated groups, and the two populations with low or no maize consumption were assumed to be the control groups, (table 20) [268].

In South Africa, serum Sa:So ratio in men and women were significantly lower in Bizana (low esophageal cancer incidence area) compared to Centane (high esophageal cancer incidence area). By contrast, urinary Sa:So ratio in men and women were significantly higher in Bizana than Centane. The estimated mean probable daily intake (PDI) in both areas (Bizana and Centane) was quite similar 6.7 to 5.8 μg FB1/ kg b.w. / day. Interestingly in both areas, urinary Sa:So ratio were significantly lower in men than in women, (table 20) [269-270].

Therefore, the authors concluded that these results negated the sphingoid bases (Sa and So) and their ratio as potential biomarker of fumonisin exposure in humans, and further studies were needed to provide a disturbance of sphingolipids metabolism in serum and urine as a biomarker of human fumonisin exposure. That conclusion was not accurate, because, strong correlation between FB1 exposure and disturbance of sphingolipids metabolism was recorded in all tested animals. Also, the disturbance of sphingolipids metabolism was more pronounced when animals were exposed to FB1 for a short period rather than a long period in the case of rats, ducks and turkeys. Furthermore, absent correlation between FB1 intake and Sa:So in serum and urine in south Africa did not reveal to an absence of FB1 exposure, because the carcinogenic effect of FB1 was recorded in its regions.

3. Conclusion

Assessment of Sa and Sa:So ratio alterations in tissues, serum and urine were the best specific and sensitive indirect biomarkers for exposure to fumonisins in all tested animal species for the following reasons:

- The lowest-observed-effect level (LOEL) required to increase Sa and Sa:So ratio was usually lower than LOEL of hepatotoxicity in all animal species.
- Sphinganine and Sa:So ratio was elevated rapidly and remained elevated in different body compartments for several days (8 days) post-intoxication.

From other side, biomarker of FB1 effects (indirect biomarkers) was dependent on many factors, such as:

- **Dose dependency:** the required dose of FB1 to increase Sa and Sa:So in serum were higher than in the tissues (liver and kidneys)
- **Organs:** Sa:So ratio in kidneys of rat and ducks were more sensitive than that in their liver. Concurrently, it was quite similar in turkeys.
- **Species:** mice were more resistant than rats, broilers and turkeys were more resistant than ducks to FB1 exposure.
- **Strains:** Sa:So ratio in liver and hepatotoxicity parameters were more sensitive in male Sprague-Dawley rats than male F344 rats and in male Mule ducks than in male Pekin ducks.
- **Sex:** Sa:So ratio in kidneys and urine was more sensitive in male F344 rats than in female F344 rats. By contrast, Sa:So ratio in liver was more sensitive in female F344 rats than in male F344 rats.
- **Duration of exposure:** fumonisins had a strong effect on sphingolipids metabolism and hepatotoxicity when animals were exposed over a short period rather than a longer period. That reveals to adaptation of animal cells to FB1 toxicity after a long exposure period.

In conclusion, sphingolipids parameters are the best specific and sensitive biomarker to FB1 exposure in animals. By contrast, in the case of humans, the authors suggested that direct detection of urinary FB1 is better biomarkers to fumonisin exposure than sphingolipids parameters.

CHAPTER 2: MATERIALS AND METHODS

This part will present the material and methods which used in FB2-toxicokinetic, and then the FBS -toxicity studies.

I. Safety handling procedures

To control hazard effects of FBs, several of safety procedures have been taken as following to: wearing protective clothing (rubber gloves and laboratory coats) was during animals and laboratory manipulations. Decontamination of laboratory tools by soaked in sodium hypochlorite (5% w/v) for at least 30min, followed by addition of acetone (5% v/v) for 30 min, and then rinsed with distilled water. Laboratory biological wastes were collected in hygienic garbage.

II.FB2-Toxicokinetic Experimental

The pharmacokinetic of FB2 in rat was already described by Shephard [28]. The objectives of our study were to describe a validation method for extraction of FB2 in plasma, and to evaluate the toxicokinetic parameters of the toxin in duck and turkey.

1. Chemicals and apparatus

1.1. Chemicals

Standard of FB2 that used for analytical purpose was purchased from Biopure (Tullin, Austria), while standard of FB2 used in animal experimentation was purchased from Sigma (St. Louis, MO, USA).

OPA (ortho-phthalaldehyde) and other chemicals of the highest quality were purchased from Sigma (St. Louis, MO, USA). HPLC grade solvents were supplied by Scharlau (Barcelone, Spain).

1.2. Apparatus

The HPLC system used for FB2 determinations was composed as follows: an AL 728 auto-sampler (Alcott micrometrics, Norcross, USA), a M 2200 pump (Bischoff, Leonberg, Germany) and a RF 10A XL programmable fluorescence detector

(Shimadzu, Japan). The chromatograms obtained were exploited using PIC 3 software from ICS (Toulouse, France).

1.3. Materials

Table 21: Material used in FB2-Toxicokinetic experimental

Name	Model	Manufacturer	Origin
Balances	AJ 100 classe 1	Mettler	Viroflay, France
	BP2100	Sartorius	Toledo, Espagne
pH-meter	522	Technalab	Toulouse, France
Centrifuge	GP 200 SX	Jouan	France
Magnetic agitator	Rotamag 10	Prolabo	France
Stir table	HS 501	Ika Labortechnik	Allemagne
Extraction vacuum	Visiprep 12 ports	Supelco	USA
Vacuum air pump	DAA-124-ED	MFG.Corp.	USA
Teflon Potter	OT13	Braun	Germany
Ultraturrax	TP18	Ika	Germany

2. Animals and sampling protocols

All experimental procedures using birds were in accordance with the French National Guidelines for the care and use of animals for research purposes. Five male mule ducks and five male turkeys (body weight around 2 kg) were experimented.

Each bird received a single IV dose of 1 mg FB2 (in NaCl 0.9%)/kg b.w. under a volume of injection of 1 ml/kg body weight in Jugular vein. Blood samples (2 ml) were taken at different times after injection of the toxin: 0, 3, 10, 20, 30, 45, 60, 90, 120, 240 min.

Concerning oral route, eight birds from each species received a single oral dose of 10 mg FB2 (in NaCl 0.9%)/kg b.w. under a volume of injection of 10 ml/kg b.w. Jugular vein blood samples (2 ml) were collected in EDTA tubes at different times after injection of the toxin: 0, 30, 60, 120, 180, and 300, 420 and 600 minutes.

All blood samples were centrifuged (15 min-3000g) and plasmas were stored at -20°C until use.

3. Extraction of FB2

Protein was precipitated by mixing 250 μ l of plasma with 500 μ l of borate buffer (0.1M, pH 5.8) and 750 μ l of acetonitrile. Samples were placed on a stir table for 30 min at 300 rpm and then centrifuged for 15 min at 3000 x g at room temperature. The supernatant fraction was then defatted by solid phase extraction on LC18 columns (Supelclean, Supelco, USA). Before that, LC18 columns were preconditioned with 3 ml of: H₂O, acetonitrile, acetonitrile/borate buffer 0.1 M, pH 5.8 (V/V), respectively. The deproteinized supernatants of the samples were passed through the LC18 columns. 4 ml of borate buffer (ph 5.8), and 3 ml of acetonitrile were used to washed the columns.

All the eluates samples were collected (around 8 ml), vortexes, and then purified by strong anion exchange (SAX) solid phase extraction (Bond Elut, Varian, Harbor City, USA). Before that, SAX columns were preconditioned with 5ml of methanol and 5 ml of methanol/H₂O (V/V). The SAX columns were then washed by 8 ml of methanol/H₂O (V/V) and 3 ml of methanol respectively. Finally, the samples were eluated by 1.5 ml of methanol/acetic acid (100/1 v/v), and evaporated at 40 °C in the dark under a gentle stream of Nitrogen. The dry residues were re-suspended with 250 μ l of acetonitrile/water (1:1) before HPLC analysis.

4. OPA derivatization and HPLC analysis

OPA (Ortho-Phtalaldehyde) reagent was prepared by mixing of 25mg OPA, 500 μ L ethanol, 25 μ l β mercaptoethanol and Borate buffer QSP 50ml, and stored at 4°C. 20 μ l of β mercaptoethanol was added into OPA reagent every 2 days in order to maintain its reaction ability, according to Rice et al [277]. Borate buffer was prepared by mixing of 3g Boric acid, 30ml distilled water, 30ml KOH1M+KOH1M QSP (pH-10.5), and distilled water QSP 100 ml.

50 μ l of OPA, 50 μ l of 0.1M borate buffer (pH 8.3), and 50 μ l of H₂O were added to 50 μ l of re-suspended sample (or standard) and finally mixed by the auto-sampler. After one minute, 20 μ l of the derivatized sample were injected in the HPLC system.

The HPLC conditions were:

Column: prontosil C18 column, 5 μ m, 250mm \times 4.6mm, 120 °A (Bischoff, Leonberg, Germany).

Mobile phase: CH₃OH/NaH₂PO₄-0.1M pH 3.35 (75/25 v/v)

Flow rate: 1.00 ml/min

Fluorescence detection: excitation and emission wavelength of 335 and 440 nm respectively.

5. Calibration curve of FB2

The calibration curve was performed to calibrate the equipments used in the experimental. FB2 standard [50.5 μ g/ml] was diluted in acetonitrile/water (1:1) to prepare ten standard solutions (0, 0.001, 0.006, 0.013, 0.025, 0.063, 0.125, 0.250, 0.650, 1.250 μ g/ml). These standards were injected in triplicate, and the average values were taken to check the relationship between the injected concentration and the observed response of the detector. The observed relation was linear between 0.0125 and 1.250 μ g/ml of FB2. The observed relation was $y = 2\text{E-}07x - 0.0033$, and the determination coefficient (R^2) was 0.9995, (figure 6).

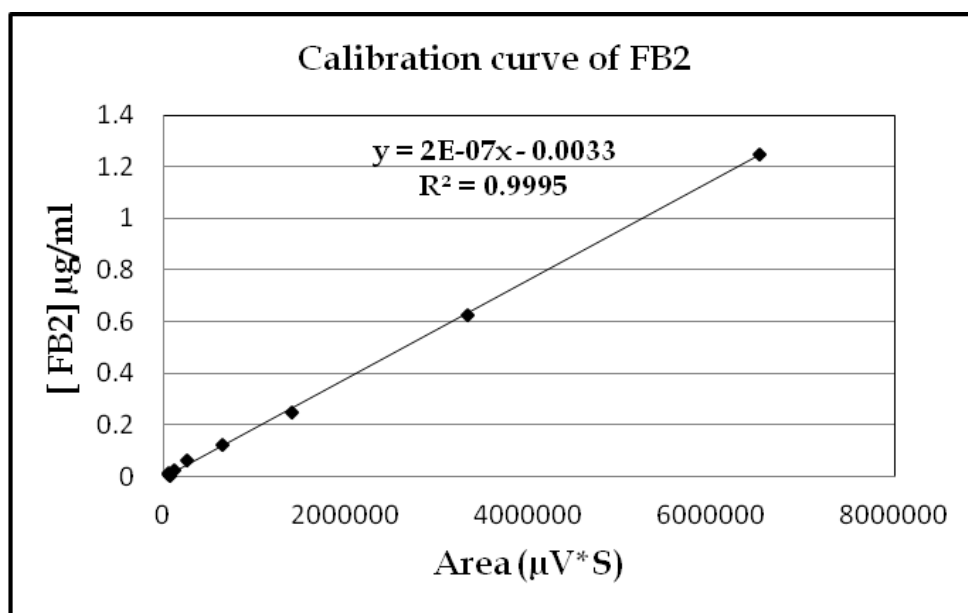


Figure 6: Calibration curve of FB2. Standards were injected in triplicate, and values were expressed as mean \pm SD

6. Validation of HPLC method of FB2

Repeatability and reproducibility conducted with standard solution [0.625 µg/ml] injected several times (n = 6) over a day, or over the week. The coefficient of variation (CV%) of repeatability and reproducibility were 3.79 and 5.46 % respectively. These results were satisfactory because the CV% were lower than 5%.

7. Validation of the whole method of FB2

Typical chromatogram obtained from blank plasma of duck fortified to a final concentration of 0.250 µg FB2/ml of plasma is shown in figure 7. Although some peaks were observed in the first part of the chromatogram, they did not interfere with the FB2 retention time (around 15 min). These peaks may correspond to substances with primary amine groups that are more polar than FB2 [277].

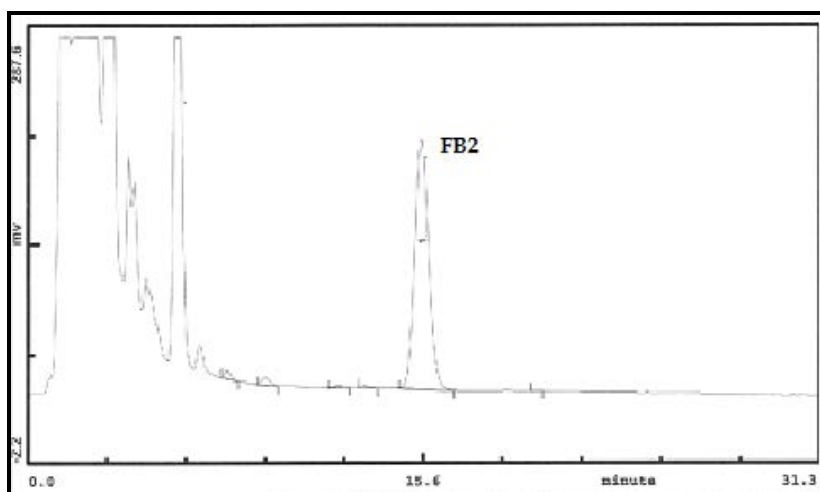


Figure 7: Typical chromatogram of FB2 was extracted from blank plasma of duck fortified to a final concentration of 0.250 µg FB2/ml of plasma

The linearity of the whole method was obtained from fortified plasmas with FB2 in a range of 0.025 to 0.250 µgFB2/ml (with $R^2 = 0.994$). All samples with higher FB2 concentrations must be diluted and re-analyzed to be within the range of linearity of the method. The limit of detection (LOD) was defined as the smallest FB1 amount that yielded a signal three times higher than the noise ratio obtained with blank tissues. The limit of quantification (LOQ) was then defined as the smallest amount of compound for which the method was validated with sufficient accuracy (<25% in both intra- and inter-day assay). The limit of detection (LOD) and

quantification (LOQ) were estimated at around 0.01, and 0.025 µg FB2/ml of plasma, respectively. The chromatograms of the plasma supplemented with 0.0125µg/ml were not different from blank plasma. These results agree with the results of Shephard, in which the LOD obtained by using SAX extraction for plasma was 0.020 µg/ml [28]. The mean recovery rate of FB2 from plasma for each level of contamination was 63% +/- 5. This recovery rate obtained was lower than the only one already described for plasma (80.4%) [58].

8. Data analysis

The concentrations of FB2 in plasma after IV dosing were plotted against time. The curves were fitted by non-linear least-squares analysis with the SigmaPlot Software (Systat software Inc., <http://www.systat.com/products/sigmaplot/>). The plasma curve of FB2 obtained for each animal after a single IV dose was fitted using the two-compartment models.

Toxicokinetic parameters after IV dosing were determined for each animal according to the equations described in Table 22, and expressed as mean +/- SE.

Table 22: Toxicokinetic parameters and formula of determination after IV dosing

Parameter	Abreviation	Formula*
Area under the curve	AUC	$(A/\alpha) + (B/\beta)$
Total plasma clearance	Cl	dose/AUC
Mean residence time	MRT	$(A/\alpha^2 + B/\beta^2) * (1/AUC)$
Apparent volume of distribution	Vdarea	Cl/β
Volume of the central compartment	Vc	dose/ (A+B)
Volume of distribution at the steady state	Vdss	$MRT * Cl$

* The plasma curve was fitted using the two-compartment models according to the following exponential equations: $f = A * \exp(-\alpha * x) + B * \exp(-\beta * x)$.

f is the function that describes the change in plasma concentration over time (x). A, B are mathematical coefficients; α is the rate constant for the distribution phase; β is the rate constant for the terminal elimination phase.

III. FBs-Toxicity Experimental

For memory, the objectives of this study were to compare the effects of FBs on sphingolipids metabolism in two avian species (ducks and turkeys), and to evaluate the toxicity of FBs on in each one.

1. Chemicals and apparatus

1.1. Chemicals

The standard of FB1 was purchased from Sigma (St. Louis, USA), while sphingolipids standards, such as C18 sphinganine (D-erythro dihydrosphingosine), C18 sphingosine (D-erythro sphingosine), and C20 sphinganine were provided by Matreya (Pleasant Gap, U.S.A).

The OPA (ortho-phthaldialdehyde) and other chemicals were of the highest quality and purchased from Sigma (St. Louis, USA). HPLC grade solvents were supplied by Scharlau (Barcelone, Spain). In all studies, distilled and deionized water was used.

1.2. Apparatus

The HPLC system used for FB1 and sphingolipids determinations was composed as follows: an AL 728 auto-sampler (Alcott micrometrics, Norcross, USA), a M 2200 pump (Bischoff, Leonberg, Germany) and a RF 10A XL programmable fluorescence detector (Shimadzu, Japan). The chromatograms obtained were exploited using PIC 3 software from ICS (Toulouse, France)

Materials used were already described in table 21.

2. Fumonisin production

To conduct this experiment, it was necessary to have a great quantity of toxins, due to duration of study, the weight and the number of the animals experimented. Concurrently, the price of FBs standards was higher. So we decided to produce the toxins

2.1. Fungal culture

The strain of *Fusarium verticillioides* (NRRL-3428) used for the production of FB1 was isolated in the laboratory from horses corn feed infected with ELEM in Toulouse area. The NRRL-3428 strain was stored on malt agar and re-cultured regularly. The culture medium used for the isolation and identification of *Fusarium* was PDA (Potato Dextrose Agar). Culture was carried out by plating in Petri-dishes (9 cm in diameter), and containing 17 ml of malt agar [105].

2.2. Toxins production

Maize was used as culture medium for toxins production. First, maize was manually sorted (to remove visibly moldy grain). Afterward, maize was sterilized by placed 50 g of crushed maize with 50 ml of distilled in Petri-dishes of 15 cm diameter, and then they were sterilized by using autoclave (121 ° C for 30 minutes). Sterilized maize was inoculation with *Fusarium verticillioides* (0.5 cm x 0.5 cm; cultivated age of 5-7 days in PDA). The boxes were incubated at 20 -23 ° C, for 4-5 weeks. The all boxes are dried by oven at 80 ° C for 3 hr. Then all cultures grind by mixer, and filtered by sieve. Then quantities of FBs are measured in the collected powder.

2.3. FBs extraction and dosing

According to the required quantity, culture material was dried in an oven at 80-90 ° C (90 min), and milled into fine flour. 25 g of powder were placed in glass bottle 250 ml, and extracted with 100 ml of acetonitrile-water (50/50 v/v) by using mechanical agitation on a stir table (300 rpm) overnight. The extract was filtered and concentrated by evaporation of the solvent. The FBs were diluted and justified by deionised water before quantification. Finally, FB1 was quantified by HPLC, according to Rice et al [277].

Purity of the crude extract was of 54% FB1, 8% FB2, and 9% FB3. Twenty-nine percent of the extracts were maize pigments.

The extract was diluted in deionized water before administration to birds to reach a final concentration of 2 mg FB1 + FB2/ml (the respective concentrations of FB1, FB2, and FB3 of dosing solution were of 1.742, 0.258, and 0.290 mg/ml).

3. Animals and sampling - experimental protocol

All experimental procedures using birds were in accordance with the French National Guidelines for the care and use of animals for research purposes. 50 male turkey poults of B.U.T. 9 strain (GFA Pierpont, Castelnau Montmirail, France), and 50 male mule ducks (Pygavi, Muret, France) at 15 days of age were adapted for 1 week at the experimental station ENSAT (Ecole Nationale Supérieure Agronomique de Toulouse - France) with free access to feed and water. Mycotoxins concentrations in feed, such as fumonisins B1 + B2, aflatoxin B1, ochratoxin A, zearalenone, deoxynivalenol, and T2 toxin were below the detection limits (50, 1, 2, 25, 50, and 50 µg/kg, respectively), by using ELISA kits.

At the end of adaptation phase, birds were divided into four groups of 25 birds (two control groups and two treated groups with FBs). The four groups were reared separately to avoid cross-contamination by FBs and a suitable light and feed program was used. For example, birds have a free feed access between 06:30 and 08:00 AM and between 03:00 and 05:00 PM.

Each day, all animals received per oral route a dose of 5 ml/kg b.w. of dilute culture extract : FBs free for the controls, and with a concentration of 2 mg FB1 + FB2/ml for the treated groups (1 hr after the beginning of the light program), for 21 days. That equal to a daily dose of 10 mg FB1 + FB2/kg b.w.

Weight and the feed consumption of birds were measured on days 0, 3, 7, 11, 14, 18, and 21.

Blood samples were collected from five random birds of each groups 8 hours after the administration of the dose, at day 0 (8hr), 3, 7, 14 and 21. The serum was collected from centrifuged dry blood tubes during 15 min at 3000 g and stored at - 20°C until use. All the analysis concerning biochemical parameters in serum

(Proteins, cholesterol, AST, ALT, LDH) were provided by Vitros laboratory (Issy-Les-Moulineaux, France).

The euthanasia was conducted immediately following blood sample collection by bleeding after electric narcosis to permit post-mortem investigations and tissues samples collection (liver and kidney). Tissues samples were stored at -20°C until use.

4. Fumonisin determination

4.1. Extraction of FB1

Extraction procedure and determination of FB1 in tissues was described by Tardieu (2008) [280].

4.1.1. Preparation of tissue homogenates

One gram of liver or kidney samples were first homogenised in 2 ml of distilled water with 25mg of NaCl by teflon Potter (500 rpm) and (or) an Ultra turrax TP18. Then, 2ml of acetonitrile/methanol (1:1) was added. Samples were placed on a stir table for 120 min at 300rpm, and then centrifuged for 15min at 3000×g at room temperature. The supernatant fraction was collected and stored at -20°C until use.

4.1.2. Extraction protocol

Three ml of the supernatant fraction was defatted twice with 4ml of hexane and centrifuged for 15min at 3000×g at room temperature. 2 ml of the aqueous phase were diluted with 8ml of Phosphate Buffer Saline (PBS, pH 7.4). All of The solution was passed through immunoaffinity columns as recommended by the manufacturer (FUMONIPREP, R. Biopharm, Glasgow, Scotland). The column was then washed with 10 ml of PBS. Finally, FB1 was eluted with 1.5 ml of methanol, and with 1.5ml of water, respectively. The eluate was evaporated at 40°C in the dark under a gentle stream of Nitrogen. The dry residue was re-suspended with 200µl of acetonitrile/water (1:1).

4.2. OPA derivatization and HPLC analysis of FB1

The preparation of OPA (Ortho-Phtalaldehyde) was described previously in FB2-toxicokinetic experimental.

The dried sample was re-suspended with 200µl of acetonitrile/water (1:1), and placed in an ultrasonicated bath for 10min.

Then 50 µl of samples (or standard) were placed on the autosampler plate of the HPLC system. Before injection, the autosampler added 50µl of OPA reagent, 50µl of 0.1M borate buffer at pH 8.3, and 50µl of H₂O. All the mixture was then mixed; and 20 µl of the derivatized mixture were injected in the HPLC system.

The HPLC conditions were:

Column: Prontosil C18 column, 5µm, 250mm×4.6mm, 120 °A (Bischoff, Leonberg, Germany).

Mobile phase: CH₃OH/NaH₂PO₄-0.1M pH 3.35 (75/25 v/v)

Flow rate: 1.00 ml/min

Fluorescence detection: excitation and emission wavelength of 335 and 440 nm respectively.

4.3. Calibration curve of FB1

Purified FB1 standard [51.1µg/ml] was diluted in acetonitrile/water (1:1) to prepare ten standard solutions (0, 0.001, 0.006, 0.013, 0.025, 0.063, 0.125, 0.250, 0.650, 1.250 and 2.50 µg/ml). These standards were injected in triplicate, and the average values were taken to check the relationship between the injected concentration and the observed response of the detector. The observed relation was linear between 0.001 and 2.50 µg/ml of FB1. The observed relation was $y = 1E-07x - 0.0019$, (figure 8). The determination coefficient (R^2) was 0.999.

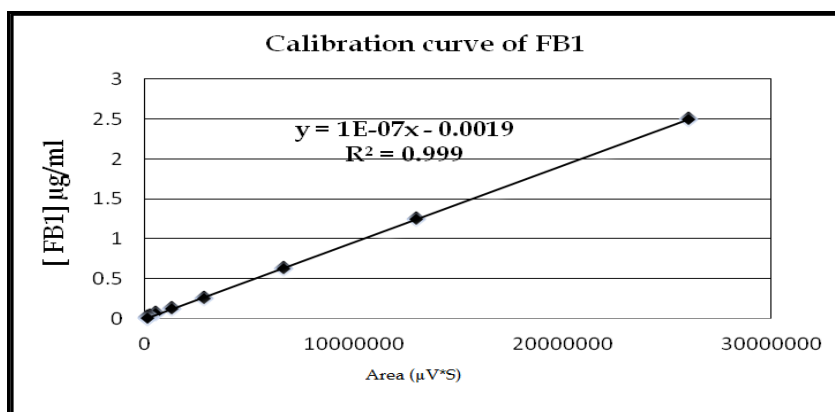


Figure 8: Calibration curve of FB. Standards were injected in triplicate, and values were expressed as mean ± SD

4.4. Validation of HPLC method of FB1

Validation of the analytical methods permits assessing analytical parameters such as repeatability and reproducibility which are important to control the dispersion during repetition of measurements.

Repeatability was conducted with two standard solutions (0.625 and 2.50 µg/ml) injected several times (n = 6) over a day. The variation coefficients (CV %) of these standard solutions were 4.09 and 4.15 % respectively. These results were satisfactory, because all of the CV% were lower than 5%. Reproducibility was suitable, because the variation coefficients of standard solutions (0.625 and 1.250 µg/ml) obtained over the course of one week (n = 6) were below 5 % (4.70 and 4.79 % respectively).

4.5. Validation of the whole method of FB1

The whole method validation was conducted with fortified blank samples. 1 g of blank tissues (liver, kidney and muscle samples) was fortified with 100µl of FB1 standard solutions (0.063, 0.125, 0.250, 0.625, 1.250 and 2.5µg/ml), to produce final samples concentration [0.0063, 0.0125, 0.025, 0.0625, 0.125 and 0.250 µg FB1/g of tissue]. No interfere peak was seen on chromatograms of fortified samples, (figure 9). The variation coefficients of repeatability and reproducibility in tissues were at range from 3.2 to 16.5 % and 2.2 to 12.5 % respectively. The limit of detection (LOD) defined as the smallest FB1 amount that yielded a signal 3 times higher than the noise ratio (obtained with blank serum or tissues) was estimated at around 0.010 µg of FB1/g for tissue. The limit of quantification (LOQ), defined as the smallest amount of the compound for which the method could quantified, was determined of 0.013 µg FB1/g of tissues. The extraction rate of FB1 was 75% for the liver and the kidney.

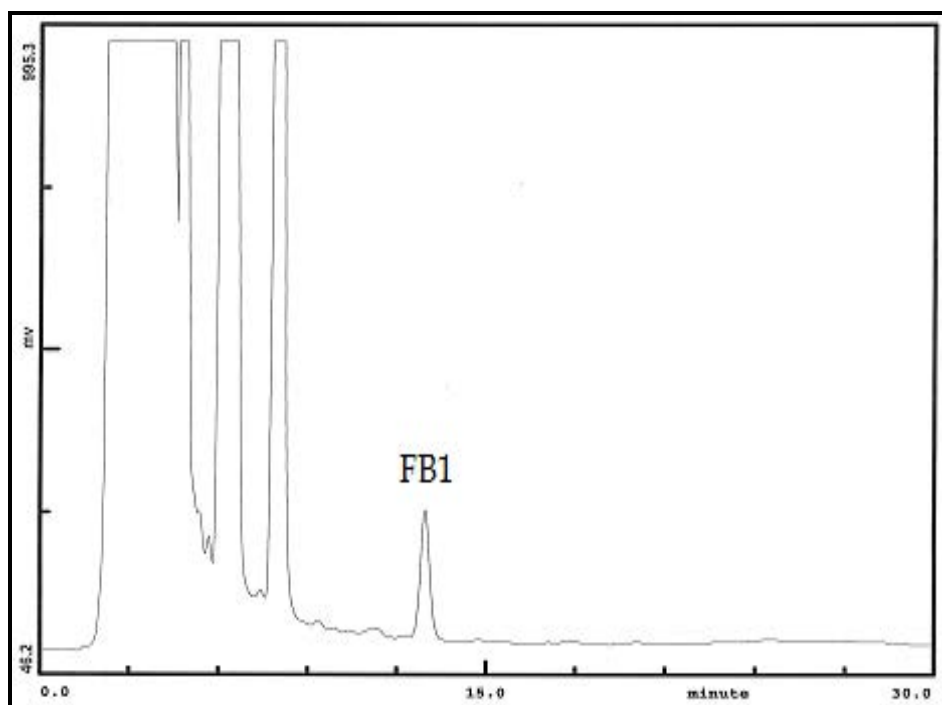


Figure 9: Typical chromatograms of FB1 was extracted from blank plasma of duck fortified to a final concentration of 0.250 μg FB1/ml of plasma

5. Sphingolipids determination

5.1. Extraction of free and phosphorylated sphingolipids

The extraction and determination procedure of sphingoid bases was described by Merrill et al. [278], and Riley et al. [279]. While, the extraction and determination procedure of sphingoid bases 1-phosphates was described by Jung-Kee Min and Hwan-Soo Yoo [281].

5.1.1. Preparation of tissue homogenates

One gram of tissue samples was first homogenised with 1ml of K buffer [200 ml of KH_2PO_4 0,1M + 800 ml of K_2HPO_4 0,1M] by using a teflon Potter (500 rpm). The Potter rinsed twice with 1ml of K buffer. The samples were then centrifuged for 15min at 3000 \times g at room temperature. The upper phase was kept and stored at -20°C until use.

5.1.2. Extraction protocol of free sphingoid bases

An internal standard (SaC20) was used to determine the percentage of extraction of sphingoid base for each sample [279].

100µL of SaC20 [10 µM] were evaporated in a glass tube. 100µL of serum or homogenate tissue, 1500µL methanol/ chloroform (4v/1v), and 100µl KOH 1M solution were added in the glass tubes containing evaporated SaC20. Adding of KOH permits basic hydrolysis to release free Sa and So from samples [283]. Samples were vortexed, and incubated at 37°C for 1hr. After cooling the samples at room temperature, 1000µl of chloroform and 2000 µl of alkaline water [100µl of NH₄OH 2N + 250 ml distilled water] were added to the samples. Tubes were gently shaken (20 times), and centrifuged for 20 min at 3000×g at room temperature. The upper alkaline aqueous phase contained sphingoid base 1- phosphates, and the lower chloroform organic phase contained free sphingoid bases. The lower chloroform phase was transferred to a new tube, and was washed with 3000µl alkaline water. This composition was centrifuged again. The new chloroform phase was dried under nitrogen at a temperature of 45-50 °C.

5.1.3. Extraction protocol of sphingoid bases 1- phosphates

In our work, according to Jung-Kee Min and Hwan-Soo Yoo [281] we have measured the amount of sphingoid bases 1 phosphate in the form of free sphingoid bases after dephosphorylation of sphingoid bases 1 phosphate by phosphatase enzyme. 100µL of SaC20 at concentration [10 µM] was evaporated in a glass tube. 100µL of serum or homogenate tissue, 250µL methanol and 0.6µl of concentrated HCl were added. The sample was placed in an ultrasonic bath for 5min in ice-cold water. The lipids were extracted by adding 500µL chloroform/NaCl 1M (1v/1v), and 25µl of NaOH-3M. The sample was vortexed for 30 s, and centrifuged for 3 min at 7500×g at room temperature. Upper alkaline aqueous phase, which contained sphingoid base 1- phosphates was kept in new glass tube. The residual sphingoid bases 1 phosphate in the lower chloroform phase were further extracted with 250µl methanol/ NaCl-1M (1v/1v) and 13µl NaOH-3M, and centrifuged again for 3 min at 7500×g at room temperature. All the upper alkaline aqueous phase fractions were collected in the same tube for each sample. Then, 130µl of reaction buffer pH 9.0 [200 mM Tris-HCl

(PH 7.4) with 75mM MgCl₂ in 2M glycine buffer] and 50 units of Alkaline Phosphatase were added to alkaline aqueous phase fractions. This mixture was mixed by vortex. 200μL of chloroform was carefully placed at the bottom of the mixture to enhance the extraction and released of free sphingoid bases after dephosphorylation of sphingoid base 1- phosphates. The tubes were sealed with parafilm and incubated at 37°C for 1hr. The dephosphorylated sphingoid bases (on free sphingoid base forms) were extracted with 300μl chloroform and 500μl methanol, and a second time with 300μl of chloroform. The lower chloroform organic phases which contained free sphingoid bases were transferred to a new tube, and washed with 3000μl of alkaline water. This composition was centrifuged again. The chloroform phase was dried under nitrogen at a temperature of 45-50 °C.

5.2. OPA derivatization and HPLC analysis of free sphingoid bases

The preparation of OPA (Ortho-Phtalaldehyde) was described previously in FB1 and FB2 determination.

The dried sample was dissolved with 20μl ethanol (or 20μl of standard), 40μl of OPA and 140μl of methanol/ water (90v/10v). All the mixture was placed in an ultrasonic bath for 10 min, centrifuged for 10 min at 3000×g at room temperature, and 20 μl was injected with the auto-sampler of HPLC.

The HPLC conditions were:

Column: Prontosil C18 column, 5μm, 250mm×4.6mm, 120 °A (Bischoff, Leonberg, Germany).

Mobile phase: CH₃OH/H₂O (90v/10v)

Flow rate: 1.20 ml/min

Fluorescence detection: excitation and emission wavelength of 335 and 440 nm respectively.

5.3. Calibration curves of free sphingoid bases

Purified Sa and So standards were diluted in ethanol to prepared a range of standard at concentrations (0.005, 0.01, 0.05, 0.125, 0.25, 0.5μM). These standards were injected in triplicate, and the average values were taken to check the relationship between the injected concentration and the observed response of the detector. The

observed relation was linear between 0.005 and 0.5 μM . The observed relationships were [Sa] $y = 2\text{E-}07x - 0.002$, and [So] $y = 2\text{E-}07x - 0.0036$. The determination coefficients (R^2) for Sa and So were 0.997 and 0.996 respectively, (figure 10).

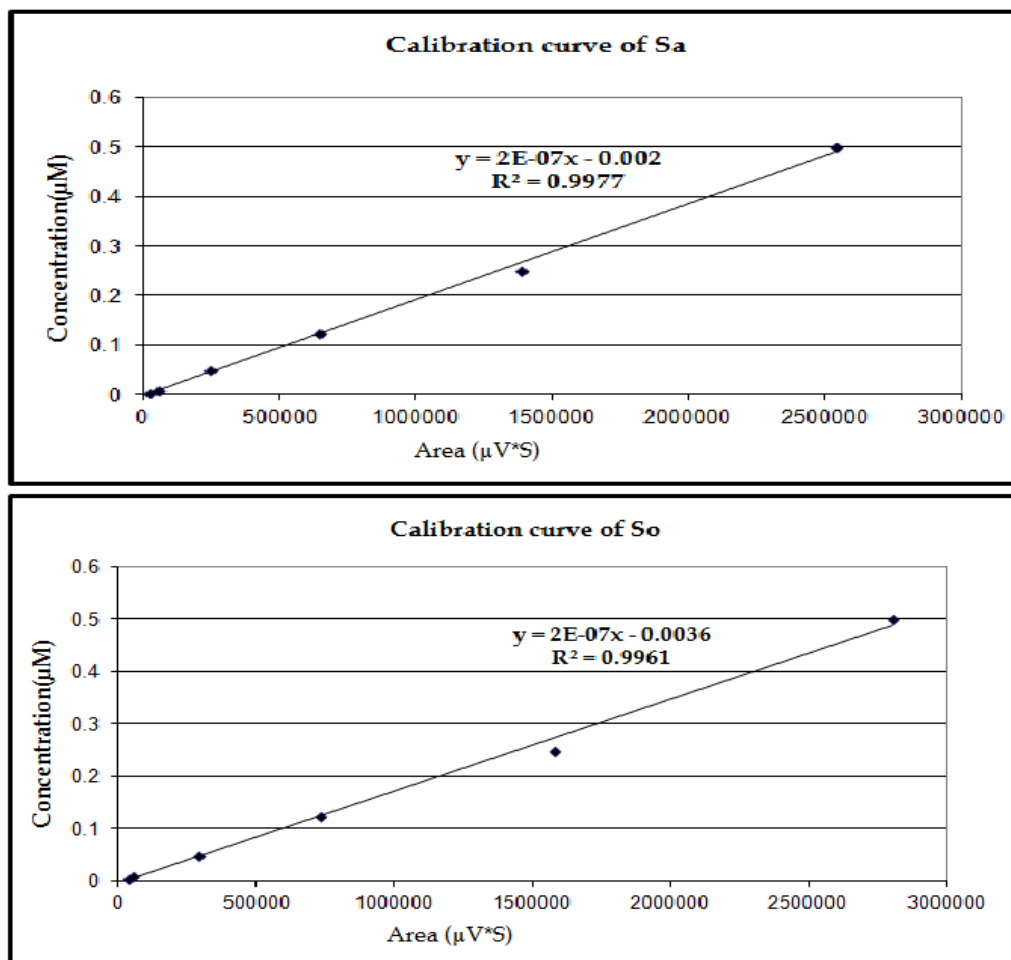


Figure 10: Calibration curve of free sphingoid bases. Standards were injected in triplicate, and values were expressed as mean \pm SD

5.4. Validation of HPLC method of free sphingoid bases

Repeatability was achieved with a mixture of two standard solutions Sa and So at concentrations (0.01 and 0.125 μM) injected several times ($n = 6$) the same day. The variation coefficient (CV %) of Sa were 2.44% and 3.47%, CV% of So were 3.75% and 3.32% respectively. The results were satisfactory because all of the CV% were lower than 5%. The reproducibility was also correct, because the variation coefficient (CV %) of mixture standard solutions Sa and So at concentrations (0.125 μM) obtained over the course of 1 week ($n=5$) was also below 5%.

5.5. Validation of the whole method of free sphingoid bases

The validation of the whole method was conducted by mixing 100 μ l of evaporated SaC20, 100 μ l of blank serum or homogenates liver obtained from ducks fed mycotoxin-free feed, with 100 μ l of mixed standards Sa and So at concentration [0.005, 0.01, 0.05, 0.125, 0.25, 0.5 μ M]. In serum, the extraction repeatability was achieved with standard solution [0.5 μ M], (figure 11). The variation coefficients (CV %) of Sa, So, SaC20 were of 8.48%, 8.01% and 4.14%, respectively. All of the CV% values are lower than 10. Moreover, reproducibility was obtained with the same standard Sa + So solution [0.5 μ M] during 1 week (n=5). The variation coefficient (CV%) of Sa, So, SaC20 were 8.25%, 7.10% and 5.53%, respectively. The average of recovery rate in serum of Sa and So was 75% \pm 8% in respect of the internal standard. In liver, the results were quite similar to those obtained in serum. The recovery rates obtained were in agreement with those obtained by M. Castegnaro, L. Garren et al for blood sample of male BDIV rats by using HPLC liquid chromatographic system to determine sphinganine and sphingosine after FB1 exposure [282]. The limit of detection (LOD) and the limit of quantification (LOQ) were estimated around 0.025 and 0.05 μ M, respectively.

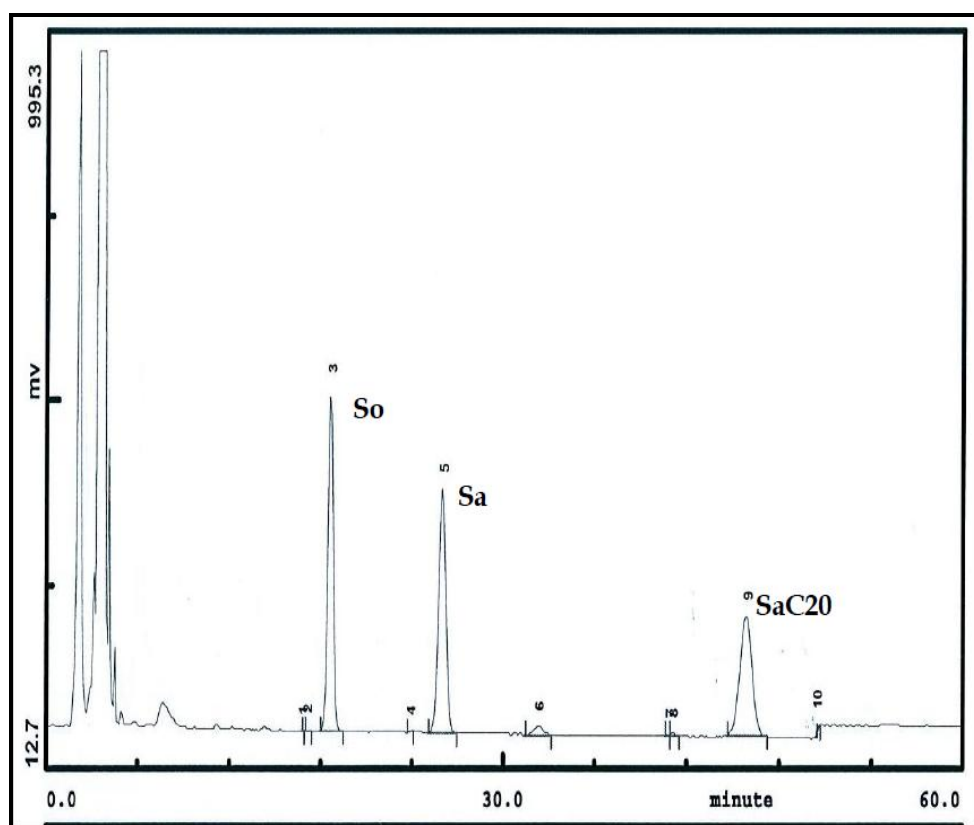


Figure 11: Typical chromatograms of mixed standards Sa and So were extracted from blank plasma of duck fortified to a final concentration of 0.25 μ M

6. Determination of oxidative stress

6.1. Preparation of homogenates tissue

Liver was rinsed with Phosphate-Buffered Saline (PBS) pH-7.4 to remove red blood cells and clots. One gram of liver was homogenised with 5 ml of K-buffer pH-7.4 [794 ml of K_2HPO_4 -0.1 M + 206 ml of KH_2PO_4 -0.1 M + 1 mM EDTA] by using a teflon Potter (500 rpm). Samples were centrifuged for 15min at 10000 \times g at - 4°C. Then, the supernatant fraction without fatty layer was collected and stored at -80°C until use.

6.2. Protein assay

The protein assay was conducted with Bio-Rad Protein Macro-assay, which was able to measure protein at range of 0.2-1.4 mg/ml of tissue homogenates as described by Bradford [296]. Bovine serum albumin (BSA) was diluted with distilled water to prepared standard solutions ranging from 0.2 to 1.4 mg/ml. 100 μ l of standard solutions or diluted samples were mixed with 5ml of diluted dye reagent

(Bio-Rad) by Vortex tubes. The mixture was incubated at room temperature for 5 min. Spectrophotometer at 595 nm was used to measure standards or samples absorbance. The protein assay was conducted three times for each sample, and the average value was considered as the final result.

6.3. Determination of catalase activity

The concentration of catalase activity was measured according to a method of L.Goth [297].

All the chemicals were purchased from Sigma (St. Louis, USA).

Na-K buffer-0.06M (pH 7.4) was prepared by mixing mono-potassium phosphate (KH_2PO_4 -0.06M) and di-sodium hydrogen phosphate (Na_2HPO_4 -0.06M), to reach pH-7.4 (1:1.5 V: V).

H_2O_2 substrate (65 $\mu\text{mole/ml}$) in Na-K buffer was prepared by mixing 0.737 ml of mother solution (H_2O_2 -30%) with 100ml of Na-K buffer. This diluted solution has to be prepared in amber flasks and is stable for to two days. Ammonium molybdate 32.4 mM was prepared in H_2O .

With L.Goth's [297] method a control was prepared by mixing 1ml of substrate H_2O_2 , 1ml of molybdate, and 0.2 ml of homogenates tissue, respectively. Two kinds of blank were realized: Blank 2 and Blank 3. For Blank 2: 1ml of substrate H_2O_2 , 1ml of molybdate, and 0.2ml of Na-K buffer were mixed respectively. For Blank 3: 1ml of Na-K buffer, 1ml of molybdate, and 0.2ml of Na-K buffer were mixed respectively. The sample tissue was treated as follows: 0.2ml of tissue homogenates sample was incubated with 1ml of substrate H_2O_2 at 37°C for 60 sec. then, the enzymatic reaction was stopped by addition of 1.0 ml molybdate. The yellow complex of molybdate and hydrogen peroxide was measured by spectrophotometer at 405 nm, against blank 3.

The results were calculated according to the formula:

$\text{CAT activity (kU/l)} = (\text{ABS sample} - \text{ABS control} / \text{ABS blank 2} - \text{ABS blank 3}) \times 271.$

Samples which exceeded 100kU/L must be diluted by Na-K buffer. The final results were expressed in U/mg of protein.

6.4. Determination of GSH activity

The concentration of GSH activity was performed by using the Ellman method (1958) [298]. All the chemicals were purchased from Sigma (St. Louis, USA).

Phosphate buffer (K buffer -0.3M, pH 7.4) was prepared by mixing mono-potassium phosphate (KH_2PO_4 -0.3M) with di-potassium phosphate (K_2HPO_4 -0.3M). The volume was justified to reach pH-7.4.

DTNB or 5, 5-dithiobis-(2-nitrobenzoic acid) (0.1M) was prepared by mixed 3.96g of DTNB with 1000ml H_2O

0.1ml of tissue homogenates sample was mixed with 0.85 ml of phosphate buffer (0.3M, pH-7.4) and 0.05 ml of (DTNB-0.01M). The GSH absorbance was read by spectrophotometer at 412 nm, against blank (0.95ml of K buffer + 0.05ml of DTNB). The GSH content in sample was calculated by using the formula:

Concentration (mole/L) = $\text{ABS}/\Sigma \times \text{L}$. Epsilon (Σ) = $14150 \text{ M}^{-1} \text{ cm}^{-1}$ according to (Eyer et al., 2003) [299]. Length of Cuve = 1cm. The final data were expressed as nmole GSH/mg of protein.

7. Serum Protein Electrophoresis (SPEP)

Serum Protein Electrophoresis (SPEP) was analysed by Saint-Gilles laboratory (la Salvetat Saint Gilles, France). Serum Protein Electrophoresis was conducted by using agarose gel electrophoresis, which purchased from Sebia.

Albumin, the largest peak, located closest to the positive electrode. The next five components (globulins) were labeled alpha1, alpha2, beta1, beta2, and gamma. The peaks for these components located toward the negative electrode, with the gamma peak being closest to that electrode (figure 12).

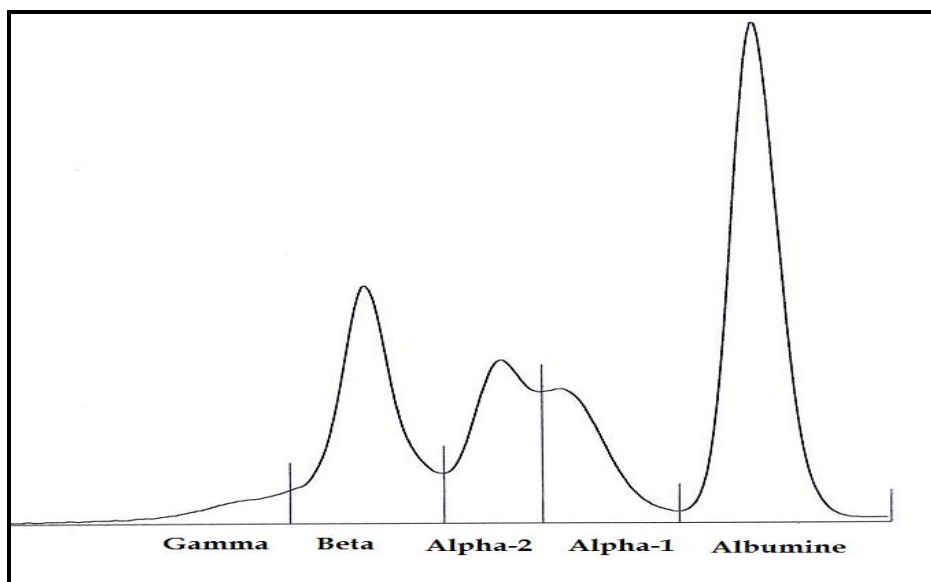


Figure 12: Typical serum protein electrophoresis for ducks by using Agarose gel electrophoresis. Samples were stained by Coomassie Blue staining. Result were obtained calculated against total amount of proteins.

8. Statistical analysis

Statistical analysis of all data was done by GraphPad Prism-5 programme. Data for all response variables were reported as means \pm SD. Two-way ANOVA was done to compare two variations in treated and control groups by species after determination of the homogeneity of variance (Hartley test). A t-test was done to compare one variation. The correlation between variables was checked by D'Agostino-Pearson test. Significant differences between controls and treated (P value < 0.05) were noted by an asterisk.

CHAPTER 3: RESULTS

I. FB2-Toxicokinetic Experimental

In the bibliography part, it was demonstrated that FBs had more toxic effect on ducks than turkeys. At the same time, FB1 was more abundant than other types of fumonisins in poultry feed. For that reason, the toxicokinetics of FB1 was studied in ducks, turkeys and laying hens, whereas toxicokinetics of other types of FBs, such as FB2 and FB3 were still unknown in these species. Therefore, it was important to study the toxicokinetics parameters of FB2 in ducks and turkeys in order to understand their different toxicity to FBs exposure.

The aim of this study was: i) to validate a method for the quantification of FB2 in plasma, ii) to reveal that toxicokinetic parameters of FB2 were different from those of FB1 in ducks and turkeys.

In all tested birds, neither mortality nor signs of pathology were observed during the toxicokinetic study. The plasma concentrations of FB2 after intravenous (IV) dosing were decreased gradually over time in both species, more rapidly in turkeys than in ducks. They were below the limit of quantification (LOQ) in turkeys and ducks after one hour and two hours post-dosing, respectively, (figure 13).

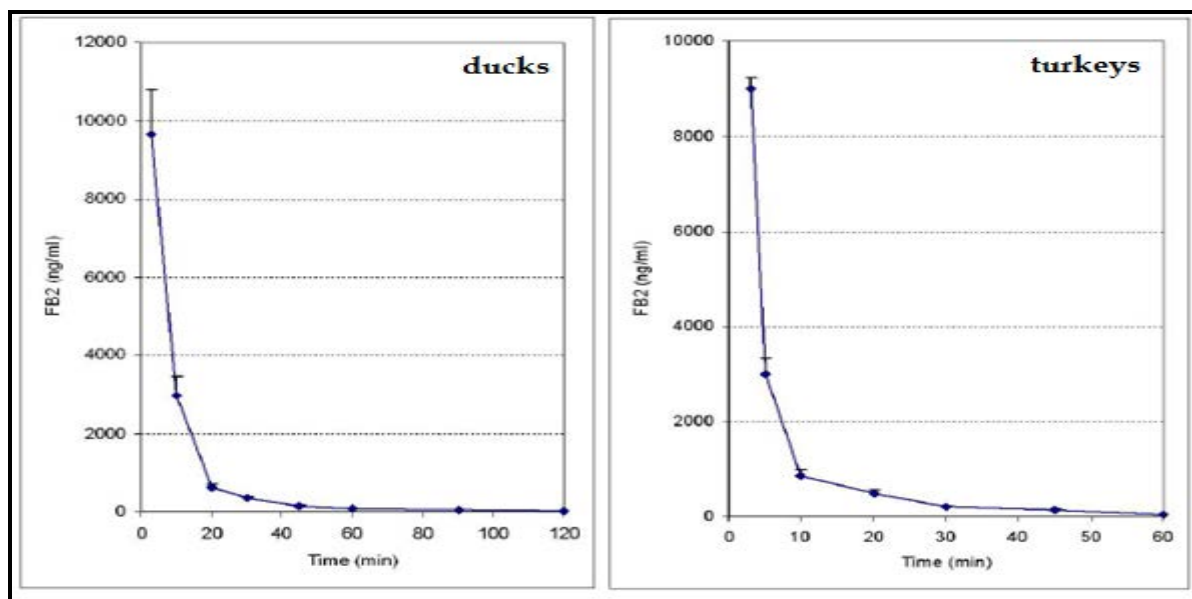


Figure 13: Plot of plasma concentration of FB2 (1 mg/kg of BW), after IV injection. Mean values \pm SE (n = 4).

The semi-logarithmic curve obtained suggests that elimination of FB2 from the plasma was biphasic in both species [an initial very rapid distribution phase (α), followed by a slower elimination phase (β)], (figure 14). Therefore, the elimination of FB2 from plasma was fitted according to a bi-exponential equation: $f(x) = A \cdot \exp(-\alpha \cdot x) + B \cdot \exp(-\beta \cdot x)$.

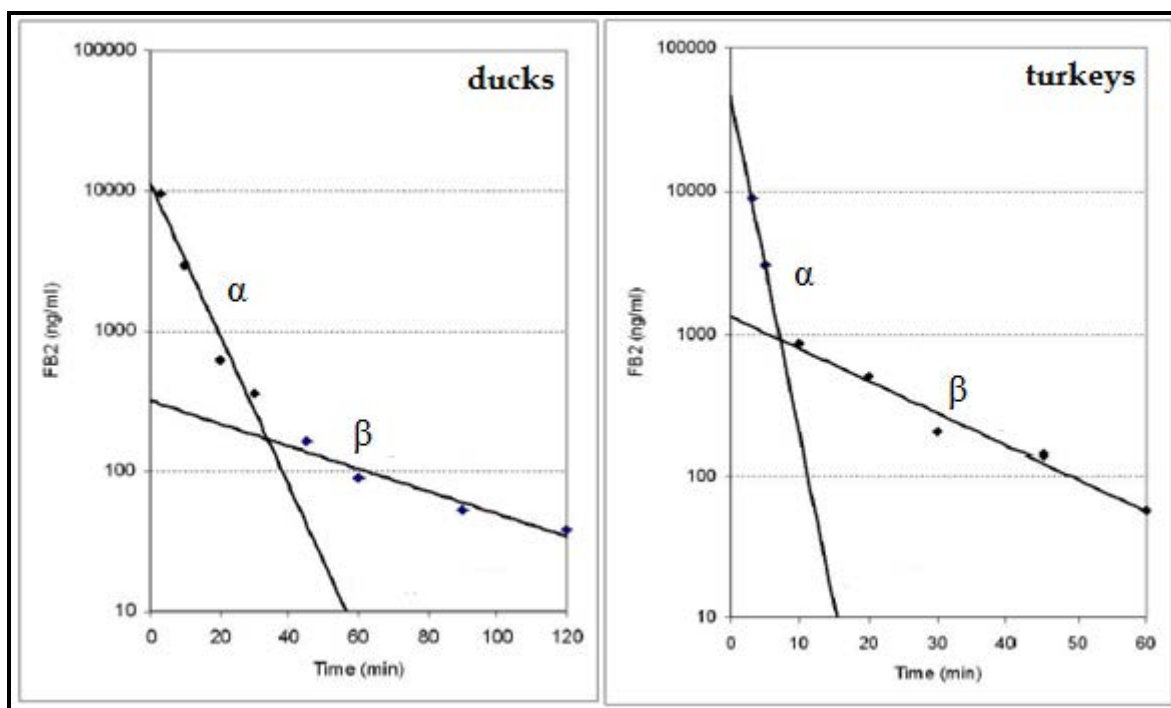


Figure 14: Semi-logarithmic plot of plasma concentration of FB2 (1 mg/kg of BW), after IV injection, showing both distributional and elimination phases. Mean values \pm SE (n = 4).

(f) is the function that describes the change in plasma concentration over time (x). A and B are mathematical coefficients; α is the rate constant for the distribution phase; β is the rate constant for the terminal elimination phase. The very high coefficient of determination (R^2) obtained from bi-exponential equation (high than 0.9995 and 0.9991 in ducks and turkeys, respectively), revealed that this model was representative of FB2 elimination from plasma in those species.

The initial half-life of distribution ($T_{1/2\alpha}$) was rapid in both species, with a faster rate in turkeys than in ducks (1 and 3.8 min, respectively). It was followed by a slower terminal phase of elimination, which was longer in ducks than in turkeys ($T_{1/2\beta} = 32$ and 12.4 min, respectively). The area under the curve (AUC) was quite

similar in ducks and turkeys (107.3 ± 4.5 and 115.47 ± 0.48 $\mu\text{g/ml/min}$, respectively). The serum clearance (Cl) of FB2 was near value in ducks and turkeys (9.3 ± 1.2 and 8.7 ± 0.7 ml/min/kg , respectively). The mean residence time (MRT) was higher in ducks than in turkeys (12.9 ± 5.1 and 5.0 ± 3.2 min). The volume of distribution (Vdarea), volume of the central compartment (Vc), and volume of distribution at the steady stage (Vdss) were higher in ducks than in turkeys. In ducks, they were 442 ± 73 , 61 ± 12 and 120 ± 32 ml/kg , respectively, whereas in turkeys, they were 154 ± 88 , 16 ± 10 and 43 ± 9 ml/kg , respectively, (table 23).

Table 23: Toxicokinetic parameters of FB2 after IV injection (1mg/kg) in ducks and turkeys

Parameter	Value (mean \pm SE)	
	Duck	Turkey
A (ng/ml)	15952 ± 198	62906 ± 3314
α (min ⁻¹)	0.18 ± 0.08	0.69 ± 0.11
B (ng/ml)	400 ± 187	1401 ± 159
β (min ⁻¹)	0.02 ± 0.01	0.056 ± 0.03
$t_{1/2\alpha}$ (min)	3.8 ± 1.6	1 ± 0.2
$t_{1/2\beta}$ (min)	32 ± 11	12.4 ± 6
AUC (ng/ml/min)	107303 ± 4439	115470 ± 477
MRT (min)	12.9 ± 5.1	5.0 ± 3.2
Cl (ml/min/kg)	9.3 ± 1.2	8.7 ± 0.7
Vdarea(ml/kg)	442 ± 73	154 ± 88
Vc (ml/kg)	61 ± 12	16 ± 10
Vdss (ml/kg)	120 ± 32	43 ± 9

A, B: mathematical coefficients; A: rate constant for the distribution phase; B: rate constant for the elimination phase; $T_{1/2\alpha}$: distribution half-life; $T_{1/2\beta}$: terminal elimination half-life; AUC: area under plasma concentration-time curve from $t = 0$ to infinity; MRT: mean residence time; Cl: total plasma clearance; Vdarea: volume of distribution; Vc: volume of the central compartment; Vdss: volume of distribution at the steady state.

After the oral administration of 10 mg/kg b.w., traces amount of FB2 was below the LOQ (25 ng FB2/ml of plasma), which were detected in plasma of ducks. Only two turkeys showed plasmatic levels of FB2 higher than the LOQ, but they were below 50 ng/ml (figure 15). The values were below 50 ng/ml cannot be fitted by exponential equation. The curves presented in figure 15 were not precise, because most of values was below the LOQ and higher then LOD (25 and 10 ng FB2/ml of plasma, respectively).

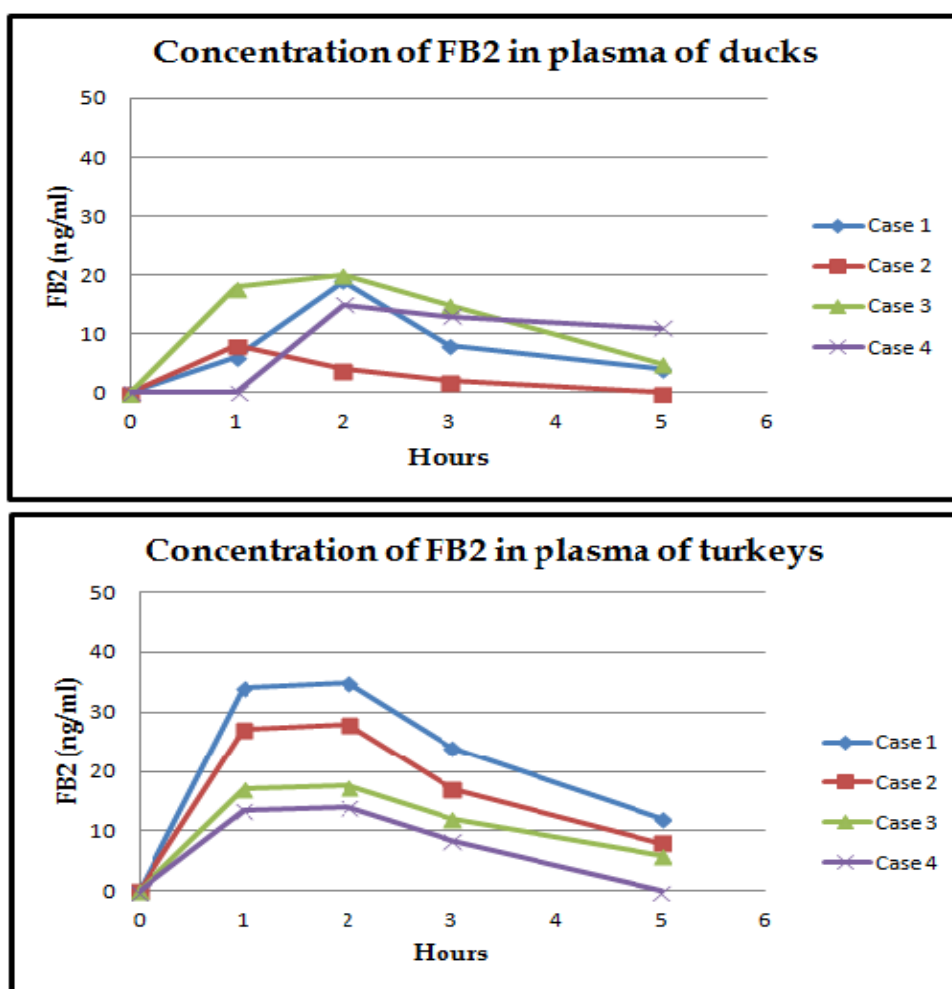


Figure 15: Plasma concentration of FB2 (1 mg/kg of BW) after oral dose. Mean values \pm SE (n = 4).

II.FBs-Toxicity Experimental

From the previous data it became clear that ducks were more sensitive to FBs toxicity than turkeys. Unfortunately, those researches were not conducted at the same time and under same conditions (age of birds, source of toxin, dose of treatment, and duration of exposure). Hence, the judgment that ducks were more sensitive to FBs toxicity than turkeys was not rigorous. In order to avoid the experimental variation and to confirm the different sensitivity between ducks and turkeys to FBs toxicity, the present study was conducted on both species at the same time and under the same conditions. Two week old birds (ducks and turkeys) received per oral route a dose of 10 mg FB1 + FB2/kg b.w. /day, for 21 days.

1. General toxicity and serum biochemistry

In treated ducks, one case of death was recorded after two weeks of exposure, and postmortem examination failed to identify cause of death. Body weight gain was significantly decreased, with high standard deviation (SD) at the end of the study by up to 0.6 kg (17% of body weight) in treated compared to control groups (Figure 16). This high standard deviation (SD) was a result of different sensitivity of ducks to FBs toxicity. That means some ducks were more affected than others to fumonisin exposure. The average of feed consumption during 21-day period was decreased in treated groups compared to control groups. In control ducks, it was 3424 g/bird during the experiment. While in treated ducks, it was 2909 g/bird. By contrast, in treated turkeys, neither mortality nor signs of illness were observed during the 21 days of exposure (Figure 16). Body weight gain did not decrease compared to the control groups. The average of feed consumption was also not affected in control and treated groups during the experiment. It was 3181 and 3056 g/bird, respectively. In both species, feed conversion ratio (FCR) was constant during the study.

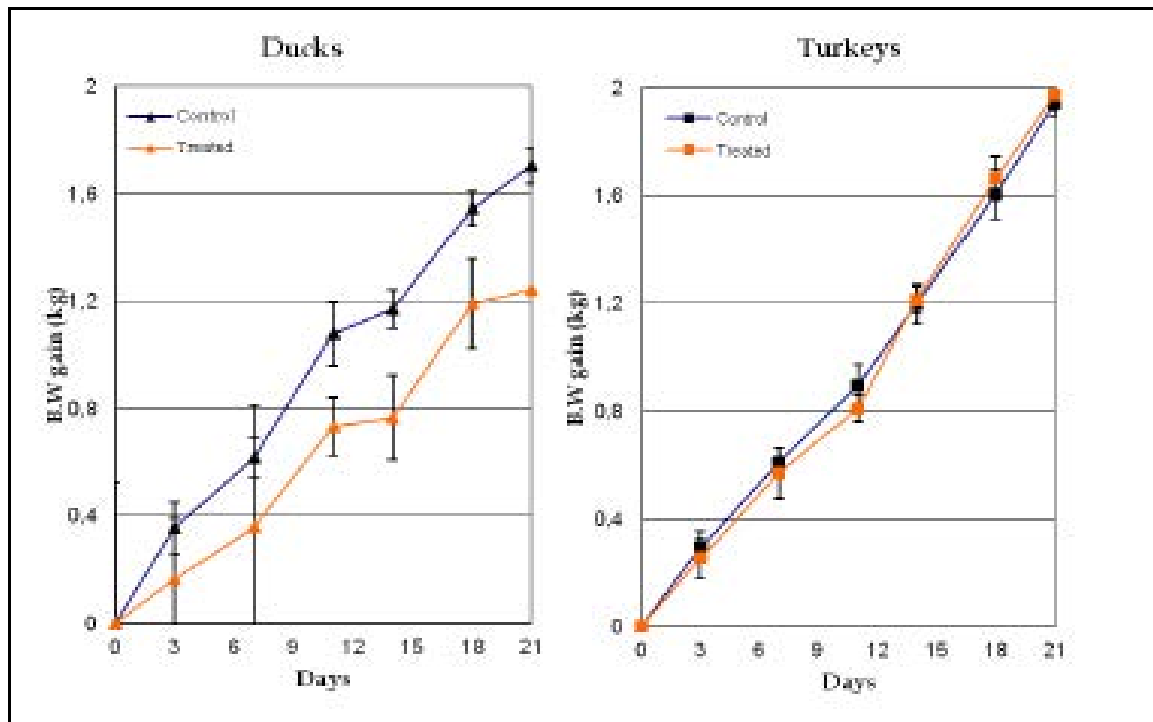


Figure 16: Effects of FBs on body weight gain in ducks and turkeys treated with 10 mg FB1 + FB2/kg b.w/day, during 21 days. Values were expressed as mean \pm SD

No macroscopic lesions were discovered by postmortem examination of tissues in all treated birds. In ducks, FB1 had increased liver weight after 7 days of exposure, while heart and gizzard weights were not affected. By contrast, in turkeys, FB1 had increased gizzard weight after 14 and 21 days of exposure, while liver and heart weights were not affected, (table 24).

Table 24: Effects of FBs on the relative organ weights

FB1 + FB2 (mg/kg b.w.)	Duck		Turkey	
	0	10	0	10
Liver (%)				
day 0	3.58 ± 0.32	3.64 ± 0.38	2.35 ± 0.30	2.26 ± 0.09
day 3	3.54 ± 0.53	3.28 ± 0.42	2.43 ± 0.30	2.26 ± 0.11
day 7	2.70 ± 0.18	3.05 ± 0.16*	2.47 ± 0.19	2.45 ± 0.37
day 14	2.49 ± 0.21	2.75 ± 0.33	2.43 ± 0.09	2.29 ± 0.13
day 21	2.27 ± 0.19	2.30 ± 0.15	2.01 ± 0.21	1.91 ± 0.12
Heart (%)				
day 0	0.77 ± 0.03	0.75 ± 0.08	0.55 ± 0.05	0.52 ± 0.06
day 3	0.70 ± 0.04	0.74 ± 0.08	0.50 ± 0.09	0.50 ± 0.07
day 7	0.69 ± 0.04	0.69 ± 0.08	0.49 ± 0.08	0.49 ± 0.03
day 14	0.64 ± 0.06	0.66 ± 0.04	0.46 ± 0.04	0.48 ± 0.05
day 21	0.66 ± 0.07	0.67 ± 0.05	0.46 ± 0.03	0.43 ± 0.02
Gizzard (%)				
day 0	3.44 ± 0.42	3.43 ± 0.38	2.17 ± 0.33	2.28 ± 0.22
day 3	3.48 ± 0.57	3.54 ± 0.32	1.92 ± 0.27	1.96 ± 0.49
day 7	3.03 ± 0.39	2.74 ± 0.27	1.95 ± 0.38	1.94 ± 0.25
day 14	2.83 ± 0.25	2.32 ± 0.29	1.63 ± 0.29	2.20 ± 0.15*
day 21	2.41 ± 0.38	2.83 ± 0.19	1.50 ± 0.27	2.05 ± 0.27*

Values were obtained from five birds per group (four birds on day 21 for ducks that received FBs) 8 hr after the first administration of FBs (day 0) and at days 3, 7, 14, and 21 and expressed as mean ± SD. *Two-way ANOVA was done to compare treated and control groups by species (significantly different, $P < 0.05$).

In treated ducks, FBs increase hepatotoxicity parameters such as protein, cholesterol, and LDH after three days of exposure until the end of the study, (table 25). Except AST which was increased only after 21 days of exposure. ALT was nearly constant during the study. By contrast, hepatotoxicity parameters in treated turkeys were not affected during the study, except an increase of AST and LDH, which significant elevated on days 14 and 7, respectively, (table 25).

Table 25: Effects of FBs on serum biochemistry

FB1 + FB2 (mg/kg b.w.)	Duck		Turkey	
	0	10	0	10
Proteins (mg/L)				
day 0	35 ± 2	37 ± 2	37 ± 2	39 ± 2
day 3	34 ± 2	40 ± 2*	36 ± 3	40 ± 2
day 7	37 ± 4	39 ± 3	38 ± 3	39 ± 5
day 14	36 ± 2	46 ± 5*	36 ± 5	36 ± 1
day 21	34 ± 2	45 ± 2*	36 ± 3	36 ± 3
Cholesterol (mmol/L)				
day 0	1.81 ± 0.20	2.01 ± 0.19	1.20 ± 0.03	1.32 ± 0.10
day 3	1.90 ± 0.12	2.31 ± 0.16*	1.09 ± 0.18	1.31 ± 0.10
day 7	2.12 ± 0.24	2.96 ± 0.45*	1.39 ± 0.16	1.27 ± 0.12
day 14	2.11 ± 0.16	4.46 ± 3.42*	1.40 ± 0.27	1.64 ± 0.15
day 21	1.72 ± 0.14	3.75 ± 1.06*	1.59 ± 0.13	1.75 ± 0.19
AST (U/L)				
day 0	46 ± 25	30 ± 9	263 ± 18	289 ± 42
day 3	24 ± 15	44 ± 20	278 ± 25	348 ± 62
day 7	23 ± 5	23 ± 16	327 ± 46	399 ± 69
day 14	37 ± 20	43 ± 16	291 ± 32	354 ± 15*
day 21	13 ± 5	56 ± 18*	289 ± 20	307 ± 37
ALT (U/L)				
day 0	18 ± 6	28 ± 3	3 ± 1	3 ± 2
day 3	21 ± 5	35 ± 13*	3 ± 1	4 ± 1
day 7	14 ± 6	27 ± 3*	4 ± 1	5 ± 2
day 14	13 ± 6	27 ± 4*	3 ± 1	3 ± 1
day 21	14 ± 5	29 ± 11*	3 ± 1	2 ± 1
LDH (U/L)				
day 0	895 ± 353	736 ± 240	1403 ± 151	1633 ± 395
day 3	524 ± 153	1639 ± 866*	1406 ± 245	2328 ± 748
day 7	448 ± 71	1058 ± 455*	1385 ± 284	3385 ± 1029*
day 14	816 ± 262	1913 ± 1069*	2231 ± 1084	3398 ± 1237
day 21	399 ± 30	3122 ± 1725*	1287 ± 306	2592 ± 595

Values were obtained from five birds per group (four birds on day 21 for ducks that received FBs) 8 hr after the first administration of FBs (day 0) and at days 3, 7, 14, and 21 and expressed as mean ± SD. *Two-way ANOVA was done to compare treated and control groups by species (significantly different, $P < 0.05$).

2. Sphingolipids alterations

Because FBs toxicity varies between ducks and turkeys, and because this toxicity could be related to disruption of the sphingolipids metabolism, we investigated the effects of FBs on the free and the phosphorylated forms of sphingolipids in tissue and serum and tried to correlate them with serum biochemistry. Results were presented, as follow:

1. Effects of FBs on amount of free sphingolipids in tissue and serum.
2. Correlation between accumulation of free sphingolipids in liver and hepatotoxicity.
3. Effects of FBs on amount of sphingolipid phosphorylated forms in liver and serum.
4. Correlation between accumulations of sphingolipid phosphorylated forms in liver and hepatotoxicity.
5. Correlation between accumulations of free sphingolipids and sphingolipid phosphorylated forms in liver

2.1. Free sphingolipids

2.1.1. Free sphingolipids in tissues

In both species, liver was less sensitive to FBs exposure than kidneys. Also, the accumulation of sphinganine in tissues was more pronounced in treated turkeys than ducks during the experiment. In livers and kidneys of treated ducks, the mean accumulation value of Sa was 14 and 42 fold higher than the control, respectively. Whereas in treated turkeys, it was 32 and 105 fold higher than the control, respectively. The concentration of sphinganine in liver increased over time during the experiment in treated groups of both species. By contrast, the concentration of sphinganine in kidneys increased from the first day (8 hr) until day 7, and then it decreased in treated groups of both species, (figure 17).

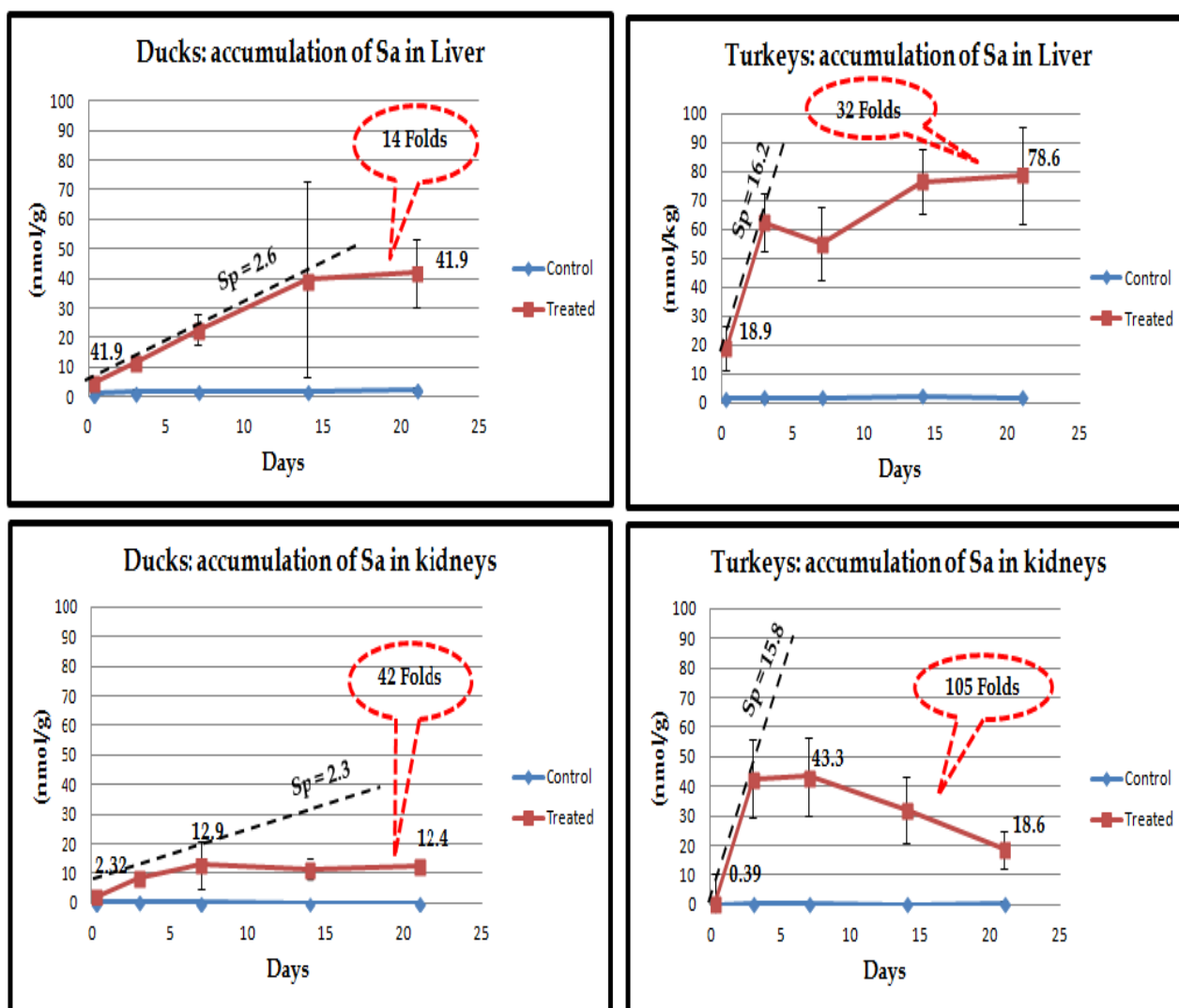


Figure 17: Effects of FBs on sphinganine in tissues in ducks and turkeys treated with 10 mg FB1 + FB2/kg b.w/day, during 21 days. Values were expressed as mean \pm SD, [Sp: slope of accumulation curve]

Interestingly in treated groups of both species, a significant increase in Sa was detected rapidly in livers and kidneys 8 hr (day 0) after first dosing. In ducks, the accumulation of sphinganine in liver and kidneys reached the maximum after 14 and 7 days, respectively. In turkeys, it reached the maximum 3 days post exposure in liver and kidneys, (figure 17 and table 26).

Table 26: Effects of FBs on sphinganine, sphingonine and Sa:So ratio in tissues

FB1 + FB2 (mg/kg BW)	Duck		Turkey	
	0	10	0	10
Sa in liver (nmol/g)				
day 0	1.10 ± 0.31	4.69 ± 1.08*	1.37 ± 0.14	18.92 ± 7.52*
day 3	1.59 ± 0.80	11.48 ± 1.59*	1.80 ± 0.74	62.23 ± 9.96*
day 7	1.78 ± 0.45	22.61 ± 5.27*	1.75 ± 0.36	55.03 ± 12.65*
day 14	1.90 ± 0.47	39.53 ± 33.04*	2.31 ± 0.49	76.42 ± 11.05*
day 21	2.32 ± 0.79	41.9 ± 11.39*	1.77 ± 0.36	78.62 ± 16.78*
So in liver (nmol/g)				
day 0	7.38 ± 3.83	7.22 ± 4.69	7.49 ± 1.37	9.03 ± 2.97
day 3	7.08 ± 1.41	7.06 ± 1.63	9.01 ± 2.49	13.19 ± 2.59
day 7	9.18 ± 3.02	9.57 ± 1.76	13.8 ± 1.75	18 ± 4.08
day 14	8.41 ± 0.91	11.6 ± 8.35	13.5 ± 2.89	17.7 ± 2.06
day 21	9.96 ± 3.13	14.2 ± 3.83	8.56 ± 4.67	17.4 ± 3.08*
Sa:So in liver				
day 0	0.16 ± 0.04	0.65 ± 0.13*	0.19 ± 0.03	2.07 ± 0.25*
day 3	0.22 ± 0.09	1.67 ± 0.3*	0.22 ± 0.04	4.78 ± 0.69*
day 7	0.2 ± 0.03	2.36 ± 0.3*	0.13 ± 0.02	3.08 ± 0.67*
day 14	0.23 ± 0.05	3.27 ± 1.28*	0.19 ± 0.07	4.31 ± 0.26*
day 21	0.23 ± 0.06	3.01 ± 0.78*	0.15 ± 0.05	4.61 ± 1.26*
Sa in kidneys (nmol/g)				
day 0	0.33 ± 0.24	2.32 ± 0.56*	0.13 ± 0.03	0.39 ± 10.08*
day 3	0.34 ± 0.07	8.35 ± 2.21*	0.22 ± 0.08	42.60 ± 13.2*
day 7	0.24 ± 0.03	12.90 ± 8.2*	0.27 ± 0.07	43.30 ± 13.3*
day 14	0.10 ± 0.02	11.50 ± 3.45*	0.09 ± 0.02	31.90 ± 11.3*
day 21	0.11 ± 0.12	12.40 ± 1.7*	0.59 ± 0.53	18.60 ± 6.5*
So in kidneys (nmol/g)				
day 0	1.71 ± 0.82	3.09 ± 1	1.36 ± 0.19	3.13 ± 0.9*
day 3	1.67 ± 0.49	3.59 ± 0.78*	1.55 ± 0.83	14.2 ± 4.1*
day 7	1.83 ± 0.38	4.59 ± 1.28*	3.1 ± 1.63	14.4 ± 1.2*
day 14	1 ± 0.19	3.39 ± 1.25*	1.16 ± 0.31	12.4 ± 3.5*
day 21	0.42 ± 0.4	3.28 ± 0.78*	1.62 ± 0.28	7.14 ± 1.6*
Sa:So in kidneys				
day 0	0.18 ± 0.03	0.77 ± 0.07*	0.09 ± 0.02	0.13 ± 0.03*
day 3	0.22 ± 0.09	2.31 ± 0.33*	0.29 ± 0.4	3.09 ± 0.85*
day 7	0.14 ± 0.05	3.33 ± 0.75*	0.22 ± 0.33	3.04 ± 1.09*
day 14	0.1 ± 0.01	3.4 ± 0.61*	0.08 ± 0.02	2.53 ± 0.96*
day 21	0.26 ± 0.07	3.89 ± 0.7*	0.37 ± 0.34	2.5 ± 0.45*

Values were obtained from five birds per group (four birds on day 21 for ducks that received FBs) 8 hr after the first administration of FBs (day 0) and at days 3, 7, 14, and 21 and expressed as mean ± SD. *Two-way ANOVA was done to compare treated and control groups by species (significantly different, $P < 0.05$).

More interestingly, the slope of sphinganine accumulation curve in liver was quite similar to the slope of sphinganine accumulation curve in kidneys in treated groups of both species. But unexpectedly, the slope of sphinganine accumulation curve in liver and kidneys were stronger in turkeys than in ducks. In treated ducks, they were 2.6 and 2.3 in liver and kidneys, respectively, while in treated turkeys, they were 16.2 and 15.8 in liver and kidneys, respectively, (figure 17). Those results revealed that the accumulation of Sa in treated ducks and treated turkeys was not tissue-dependence (liver and kidney). However, it was dependent on the different species (ducks and turkeys). Furthermore, the slope of sphinganine accumulation curve was in accordance with a more rapid accumulation of Sa in turkeys than in ducks.

In treated groups of both species, sphingosine in liver was not affected during the study, except in turkeys, where a significant increase was observed on day 21. By contrast, sphingosine in kidneys increased from day 3 until day 7 in ducks, whereas it rapidly increased from the 8th hour on day 1 until day 7 in turkeys. Afterward, their levels decreased during the study, (table 26). Sa:So ratio in tissues was rapidly elevated 8 hours post-exposure in both species, (table 26). The elevation of Sa:So ratio in liver and kidneys were dependent on the increase of Sa in those tissues, because sphingosine in liver was not affected during the study in both species, and elevation of sphingosine in duck kidneys was recorded 3 days post-dosing.

2.1.2. Free sphingolipids in serum

In treated groups, sphinganine was elevated faster in ducks than in turkeys, (8 hr and 3 days post-dosing, respectively). In control groups, the average amount of Sa in serum was quite similar in ducks and turkeys (0.144 and 0.112 μM , respectively). Whereas in treated groups, the average amount of Sa in serum was 2 times higher in ducks than turkeys (0.760 and 0.362 μM , respectively). The liberation ability of Sa was slightly similar in treated ducks and turkeys (5 and 3 folds than control, respectively), (table 27).

Table 27: Effects of FBs on free sphingolipid forms in serum

FB1 + FB2 (mg/kg b.w.)	Duck		Turkey	
	0	10	0	10
Sa in serum (μM)				
day 0	0.15 ± 0.1	0.24 ± 0.04	0.14 ± 0.06	0.13 ± 0.08
day 3	0.11 ± 0.06	0.55 ± 0.24*	0.11 ± 0.04	0.35 ± 0.12*
day 7	0.12 ± 0.08	0.38 ± 0.15*	0.13 ± 0.05	0.44 ± 0.11*
day 14	0.21 ± 0.11	1.48 ± 0.84*	0.07 ± 0.03	0.49 ± 0.11*
day 21	0.13 ± 0.06	1.15 ± 0.21*	0.11 ± 0.08	0.40 ± 0.06*
So in serum (μM)				
day 0	0.45 ± 0.32	0.33 ± 0.04	0.37 ± 0.14	0.24 ± 0.22
day 3	0.26 ± 0.12	0.39 ± 0.19	0.3 ± 0.15	0.32 ± 0.12
day 7	0.3 ± 0.16	0.22 ± 0.09	0.43 ± 0.23	0.38 ± 0.06
day 14	0.55 ± 0.26	0.73 ± 0.34	0.19 ± 0.07	0.39 ± 0.08
day 21	0.33 ± 0.11	0.59 ± 0.12*	0.34 ± 0.19	0.35 ± 0.05
Sa:So in serum				
day 0	0.3 ± 0.06	0.73 ± 0.14*	0.36 ± 0.04	0.62 ± 0.26*
day 3	0.11 ± 0.06	0.55 ± 0.24*	0.11 ± 0.04	0.35 ± 0.12*
day 7	0.4 ± 0.07	1.72 ± 0.05*	0.31 ± 0.04	1.18 ± 0.24*
day 14	0.38 ± 0.05	1.97 ± 0.2*	0.35 ± 0.02	1.26 ± 0.11*
day 21	0.38 ± 0.05	1.95 ± 0.16*	0.3 ± 0.07	1.15 ± 0.08*

Values were obtained from five birds per group (four birds on day 21 for ducks that received FBs) 8 hr after the first administration of FBs (day 0) and at days 3, 7, 14, and 21 and expressed as mean ± SD.*Two-way ANOVA was done to compare treated and control groups by species (significantly different, $P < 0.05$).

As for the liver, the amount of Sa in serum was increased with the duration of the exposure in treated groups of both species. On day 3, the amounts of Sa in treated ducks and turkeys were significantly increased: 5 and 3 times higher than control groups, respectively. On day 21, the amount of Sa in treated ducks and turkeys was significantly increased: 9 and 4 times higher than control groups, respectively, (table 27).

In treated groups of both species, no significant elevation of sphingosine was recorded in the serum of treated groups as compared with control groups. Except for day 21 when an elevation of So two times higher in treated ducks than control ones, was recorded, (table 27).

Significant elevation of Sa:So ratio was recorded from the 8th hr post-dosing until the end of the study in treated groups of both species. Concurrently, the increase of Sa and decrease of So were not significant at the 8th hr of exposure in treated groups. This phenomenon proven that: Sa:So ratio was highly sensitive to FBs exposure, (table 27).

The accumulation of Sa in treated ducks livers showed a significant correlation ($P < 0.05$) with hepatotoxicity parameters, such as protein ($R^2 = 0.4699$), cholesterol ($R^2 = 0.7535$) and LDH ($R^2 = 0.5206$). By contrast, no correlation was observed between accumulation of Sa in liver of turkeys and hepatotoxicity. Only cholesterol was slightly correlated with Sa accumulation in liver ($R^2 = 0.4086$), (figure 18 and table 28).

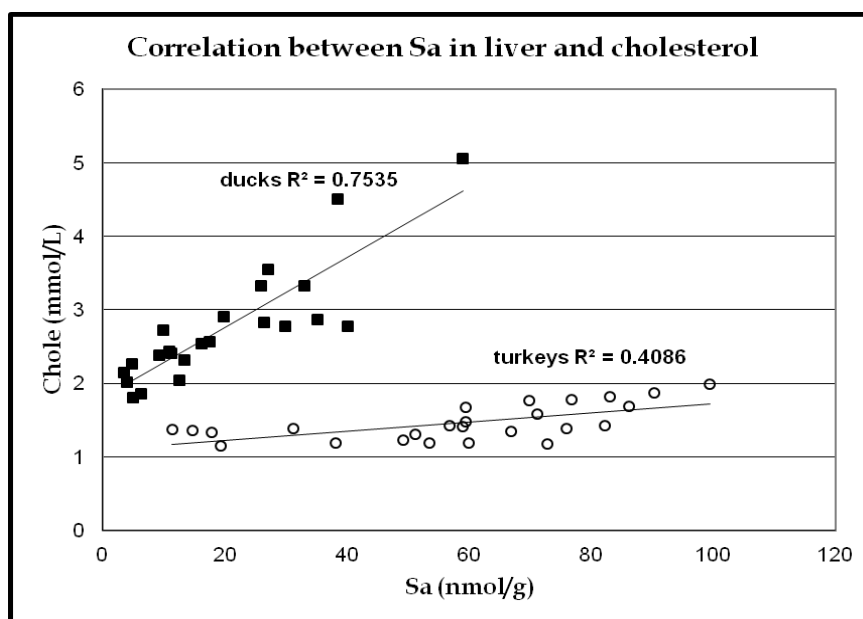


Figure 18: Correlation between Sa in liver and cholesterol in treated groups. [(R^2) was linear regression. D'Agostino-Pearson test was done to check the correlation between variables (significantly correlation, $P < 0.05$)].

Table 28: Correlation between Sa in liver and hepatotoxicity parameters

Objects	Treated ducks (R ²)	Treated turkeys (R ²)
Sa in liver / proteins	0.4699*	0.0215
Sa in liver / cholesterol	0.7535*	0.4086*
Sa in liver / AST	0.1570	0.1033
Sa in liver / ALT	0.0936	0.0025
Sa in liver / LDH	0.5206*	0.1802

(R²) linear regression.* D'Agostino-Pearson test was done to check the correlation between variables (significantly correlation, $P < 0.05$).

Interestingly, the quantity of Sa in serum was dependent on the quantity of Sa in liver, (figure 19). This result was concluded from the significant correlation obtained between the amount of Sa in liver and serum in both treated groups (in ducks $R^2 = 0.6370$, and in turkeys $R^2 = 0.7201$). However, the equation of the correlation was different between the species, (figure 19). Also Sa:So ratio in serum showed strong significant correlation with Sa:So ratio in liver of ducks and turkeys ($R^2 = 0.8849$ and 0.8316 , respectively) ($P < 0.001$).

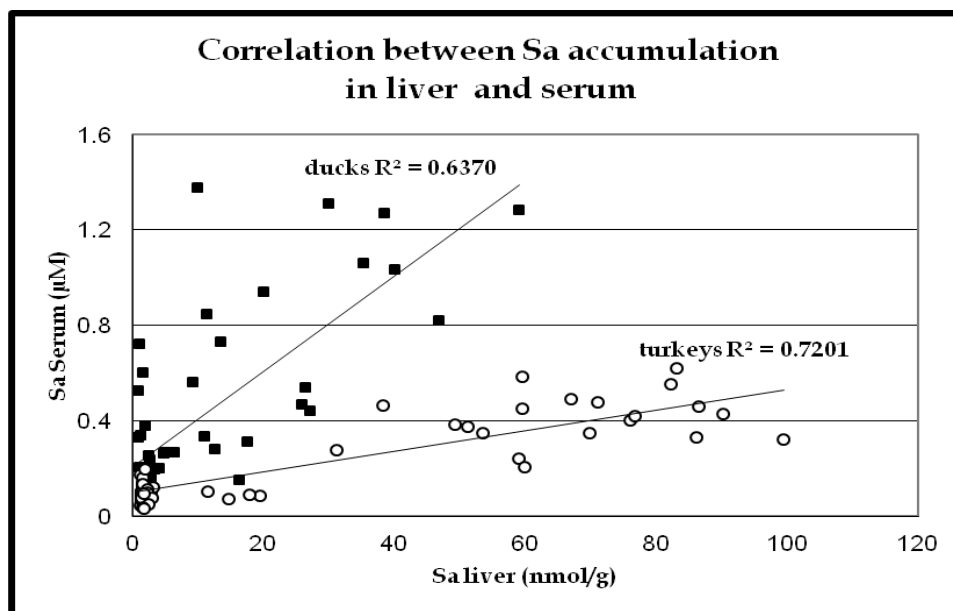


Figure 19: Correlation between Sa in liver and Sa in serum of treated groups.

[(R²) was linear regression. D'Agostino-Pearson test was done to check the correlation between variables (significantly correlation, $P < 0.05$)].

2.2. Sphingolipid phosphorylated forms

Because, the amount of Sa was higher in turkey livers than duck livers, the amount of Sa in serum was higher in ducks than turkeys, the accumulation of Sa in duck livers was correlated with hepatotoxicity, while the accumulation of Sa in turkey livers was not correlated with hepatotoxicity. The investigations done on free sphingolipids could not explain the different toxicity between ducks and turkeys to FBs exposure. Therefore, the effects of FBs on sphingolipid phosphorylated forms in liver and serum, and the effects of sphingolipid phosphorylated forms on hepatotoxicity were studied in both species to answer their different toxicity to FBs exposure.

2.2.1. Sphingolipid phosphorylated forms in liver

The phosphorylation ability of free sphingoid bases in the presence of FBs was tested in ducks and turkeys to answer their different toxicity to FBs exposure.

The phosphorylation ability was quite similar in ducks and turkeys (8 and 6 times higher than control groups, respectively). However, the average amount of Sa1P was 3 times higher in turkeys than in ducks in both groups (control and treated). Interestingly, in treated groups, significant elevation of Sa1P rapidly appeared 8 hr post-dosing, but Sa1P reached the maximum level faster in turkeys than in ducks (3 and 7 days post-dosing). Those results were in accordance with slope of Sa1P accumulation curve in liver, which was stronger in treated turkeys than in treated ducks (177 and 36, respectively), (figure 20, and table 29)

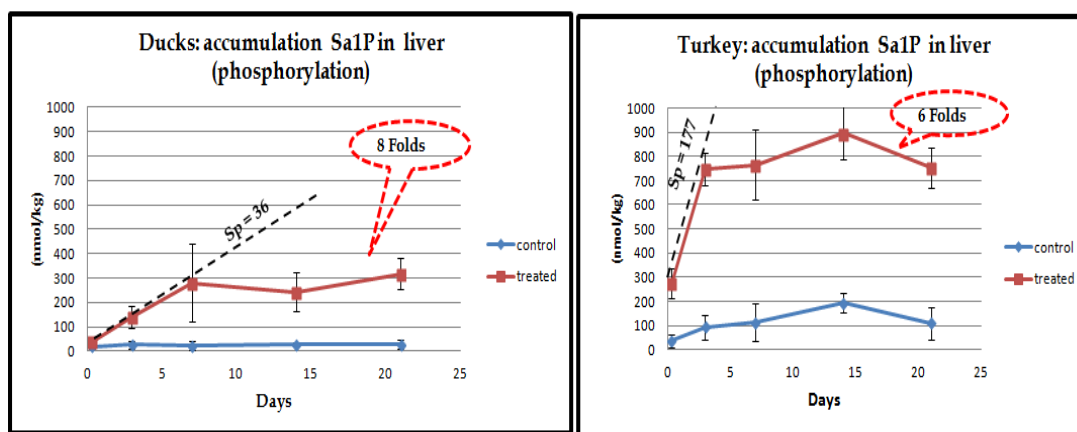


Figure 20: Effects of FBs on Sa1P in liver of ducks and turkeys treated with 10 mg FB1 + FB2/kg b.w/day, during 21 days. Values were expressed as mean \pm SD, [Sp: slope of accumulation curve]

Table 29: Effects of FBs on sphingolipid phosphorylated forms in liver

FB1 + FB2 (mg/kg b.w.)	Duck		Turkey	
	0	10	0	10
Sa1P in liver (nmol/kg)				
day 0	18.96 \pm 6.5	37.52 \pm 15.99*	37.45 \pm 26.72	274.85 \pm 60.61*
day 3	28.02 \pm 15.23	142.48 \pm 45.58*	94.29 \pm 51	749.27 \pm 67.62*
day 7	23.64 \pm 17.17	280.62 \pm 159.73*	113.51 \pm 78.71	764.03 \pm 144.58*
day 14	28.05 \pm 9.48	242.89 \pm 79.94*	194.62 \pm 40.67	895.22 \pm 110.06*
day 21	29.09 \pm 17.30	318 \pm 63.73*	109.97 \pm 66.94	753.27 \pm 84.05*
So1P in liver (nmol/kg)				
day 0	99.66 \pm 55.15	156.67 \pm 141.7	149 \pm 93.3	253.75 \pm 57.86
day 3	96.16 \pm 46.21	98.97 \pm 47.78	195.45 \pm 127.84	298.78 \pm 144.79
day 7	94.54 \pm 88.56	197.89 \pm 124.02	313.09 \pm 259.07	533.62 \pm 300.14
day 14	45.47 \pm 13.57	185.59 \pm 54.28*	306.68 \pm 133.55	345.51 \pm 83.3
day 21	87.72 \pm 58.51	209.52 \pm 62.26*	180.16 \pm 89.14	284.77 \pm 19.36*
Sa1P/So1P in liver				
day 0	0.25 \pm 0.12	0.38 \pm 0.25	0.25 \pm 0.11	1.13 \pm 0.31*
day 3	0.36 \pm 0.22	1.74 \pm 0.93*	0.53 \pm 0.18	2.78 \pm 0.68*
day 7	0.39 \pm 0.4	1.34 \pm 0.33*	0.39 \pm 0.08	1.84 \pm 0.89*
day 14	0.63 \pm 0.13	1.33 \pm 0.33*	0.69 \pm 0.18	2.64 \pm 0.26*
day 21	0.35 \pm 0.12	1.39 \pm 0.36*	0.76 \pm 0.20	2.65 \pm 0.34*

Values were obtained from five birds per group (four birds on day 21 for ducks that received FBs) 8 hr after the first administration of FBs (day 0) and at days 3, 7, 14, and 21 and expressed as mean \pm SD.*Two-way ANOVA was done to compare treated and control groups by species (significantly different, $P < 0.05$).

Concerning So1P a significant elevation was recorded 14 and 21 days after exposure in ducks and turkeys respectively. The average amount of So1P was 2 times higher in turkeys than in ducks, but the phosphorylation ability was similar in both species (2 times higher than control groups), (table 29).

Sa1P:So1P ratio was significantly increased 8 hours and 3 days post-dosing in turkeys and ducks, respectively, (table 29). This result revealed that elevation of Sa1P:So1P ratio in liver was dependent on elevation of Sa1P, which increased rapidly before the increase of So1P in both species

2.2.2. Sphingolipid phosphorylated forms in serum

The Sa1P increased after 3 days post-dosing in both species. In control groups, the average amount of Sa1P was similar in ducks and turkeys (0.233 and 0.277 μ M, respectively), whereas in treated groups, it was 1.5 times higher in ducks than in turkeys (1.033 and 0.803 μ M, respectively), (table 30).

Table 30: Effects of FBs on sphingolipid phosphorylated forms in serum

FB1 + FB2 (mg/kg b.w.)	Duck		Turkey	
	0	10	0	10
Sa1P in serum (μM)				
day 0	0.25 \pm 0.16	0.32 \pm 0.13	0.49 \pm 0.19	0.25 \pm 0.19
day 3	0.23 \pm 0.06	1.34 \pm 1.14*	0.29 \pm 0.09	0.84 \pm 0.50*
day 7	0.27 \pm 0.18	0.99 \pm 0.19*	0.21 \pm 0.03	0.98 \pm 0.40*
day 14	0.20 \pm 0.06	1.73 \pm 0.82*	0.18 \pm 0.02	1.13 \pm 0.21*
day 21	0.23 \pm 0.13	0.79 \pm 0.43*	0.21 \pm 0.14	0.82 \pm 0.07*
So1P in serum (μM)				
day 0	0.53 \pm 0.37	0.47 \pm 0.43	1.91 \pm 0.76	0.48 \pm 0.35*
day 3	0.49 \pm 0.15	0.48 \pm 0.13	0.40 \pm 0.09	0.61 \pm 0.38
day 7	0.65 \pm 0.43	0.56 \pm 0.10	0.68 \pm 0.15	0.88 \pm 0.32
day 14	0.45 \pm 0.18	0.37 \pm 0.20	0.37 \pm 0.12	0.56 \pm 0.25
day 21	0.78 \pm 0.58	0.40 \pm 0.14	0.82 \pm 0.74	0.65 \pm 0.41
Sa1P/So1P in serum				
day 0	0.48 \pm 0.04	0.90 \pm 0.41*	0.25 \pm 0.01	0.50 \pm 0.09*
day 3	0.50 \pm 0.19	2.53 \pm 1.67*	0.73 \pm 0.11	1.49 \pm 0.76*
day 7	0.44 \pm 0.14	1.81 \pm 0.53*	0.32 \pm 0.06	1.10 \pm 0.09*
day 14	0.44 \pm 0.07	6.98 \pm 6.88*	0.51 \pm 0.10	2.78 \pm 2.43
day 21	0.36 \pm 0.13	1.89 \pm 0.39*	0.31 \pm 0.07	2.22 \pm 2.33

Values were obtained from five birds per group (four birds on day 21 for ducks that received FBs) 8 hr after the first administration of FBs (day 0) and at days 3, 7, 14, and 21 and expressed as mean \pm SD.*Two-way ANOVA was done to compare treated and control groups by species (significantly different, $P < 0.05$).

The liberation ability of Sa1P was quite similar in ducks and turkeys (4 and 3 times higher than control, respectively), (table 30).

The amount of So1P in the serum of control groups was not different to the treated groups in both species, (table 30). Sa1P:So1P ratio was highly sensitive to FBs exposure, which was elevated 8 hr post-exposure in both species, (table 30).

The amount of Sa1P in liver was strongly significantly correlated with the amount of Sa in liver in treated groups of both species (in ducks $R^2 = 0.8813$, and in turkeys $R^2 = 0.8856$) (P value < 0.001), (figure 21).

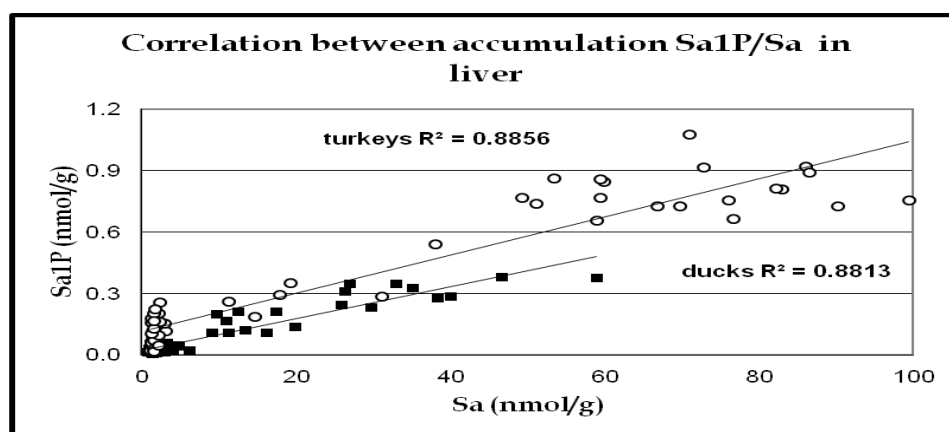


Figure 21: Correlation between Sa1P and Sa in liver of treated groups. [(R^2) was linear regression. D'Agostino-Pearson test was done to check the correlation between variables (significantly correlation, $P < 0.05$)].

Interestingly, the equation of correlation was very close in ducks and turkeys. This result demonstrated that the amount of Sa1P in liver was very dependent on the amount of Sa in liver. Moreover, the accumulation of Sa1P in liver was not correlated with hepatic toxicity parameters in both species, nor with Sa1P in serum (in ducks $R^2 = 0.368$, and in turkeys $R^2 = 0.5501$), (table 31).

Table 31: Correlation between Sa1P in liver and hepatotoxicity parameters

Objects	Treated ducks (R^2)	Treated turkeys (R^2)
Sa1P/proteins	0.2543	0.0056
Sa1P/cholesterol	0.3985	0.065
Sa1P/SAT	0.0181	0.2763
Sa1P/ALT	0.0493	0.078
Sa1P/LDH	0.1378	0.5271

(R^2) linear regression. D'Agostino-Pearson test was done to check the correlation between variables (No significantly correlation was obtained, $P > 0.05$).

No correlation was obtained between So1P and So in liver of ducks and turkeys ($R^2 = 0.0663$ and 0.2113 , respectively). This result was expected, because So in liver was not affected by FBs exposure in both species.

From the previous results it became clear that sphinganine was highly sensitive to FBs exposure in both species. In addition, the accumulation of sphinganine was implicated in hepatotoxicity in ducks, but that was not the case in turkeys. Therefore, the correlation between accumulation of FBs and free sphingolipids in liver was interesting to be investigated in both species.

The average amount of FB1 in liver was higher in treated turkeys than in treated ducks (table 32). The amount of FB1 in controls was always below the limit of quantification (13 ng/g). The level of FB2 was below the LOQ in all samples.

Table 32: Amount of FB1 in liver (ng/g) of ducks and turkeys fed 10 mg FB1+FB2/kg

	Ducks	Turkeys
day 0	35.7 ± 4.2	44.0 ± 2.3
day 3	52.9 ± 3.1	91.5 ± 3.5
day 7	43.2 ± 1.2	128.9 ± 7.4
day 14	78.1 ± 8.3	97.3 ± 6.8
day 21	113.2 ± 8.4	130.0 ± 7.6

Values were obtained from five birds per group (four birds on day 21 for ducks that received FBs) 8 hr after the first administration of FBs (day 0) and at days 3, 7, 14, and 21 and expressed as mean ± SE.

Interestingly, significant correlation was recorded between accumulation of Sa and FBs in the liver of ducks and turkeys ($R^2 = 0.7801$ and 0.7128 , respectively), but the equation of correlation was different between two species, (figure 22).

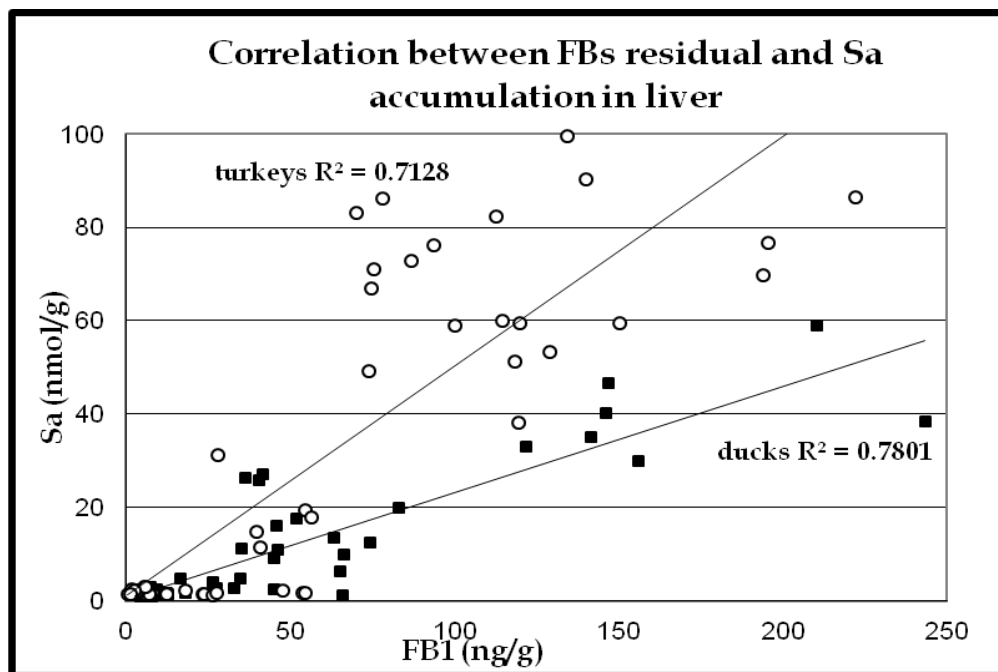


Figure 22: Correlation between Sa and FBs in liver.

[(R^2) was linear regression. D'Agostino-Pearson test was done to check the correlation between variables (significantly correlation, $P < 0.05$)].

3. Oxidative damages

The effects of FBs on free sphingolipids and sphingolipid phosphorylated forms failed to explain the different toxicity between ducks and turkeys to FBs exposure. On the other hand, oxidative damage effects of FBs have been reported in rodents [314-315-316].

Catalase (CAT) and glutathione (GSH) concentrations have been also investigated to reveal whether FBs produces oxidative damages in the liver (figure 23). Statistical analysis (t-test) of these parameters did not reveal significant difference between control and treated groups in both species.

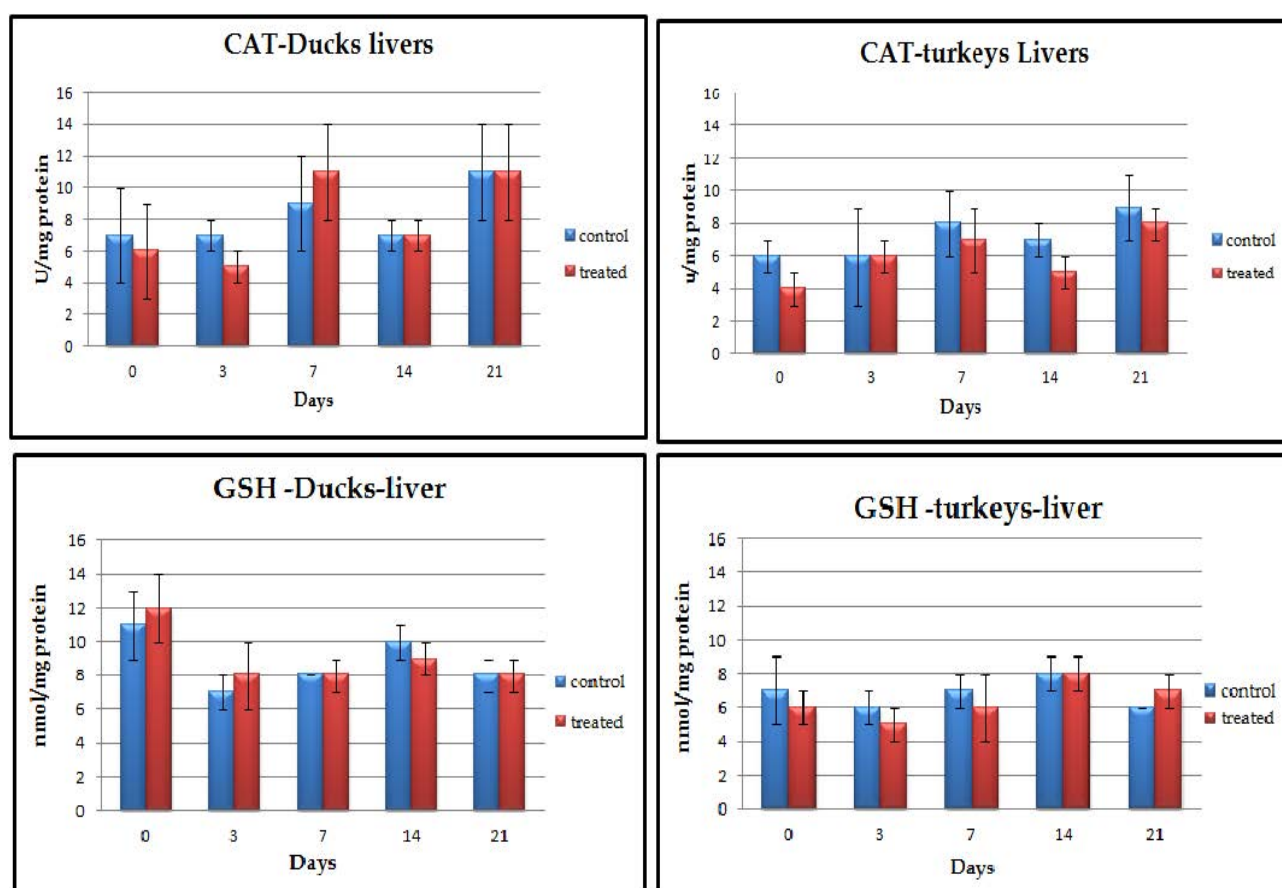


Figure 23: Effects of FBs on oxidative damage parameters in ducks and turkeys treated with 10 mg FB1 + FB2/kg b.w/day, during 21 days. Values were expressed as mean \pm SD

4. Inflammatory response

The elevation of serum total protein was recorded only in treated ducks from day 3 post-dosing until the end of the study, but not in turkeys. For that reason, it was important to induce further investigation on inflammatory proteins in ducks and turkeys as way to explain their different toxicity to FBs exposure.

Interestingly, serum protein electrophoresis analysis appeared a significant augmentation in inflammatory proteins only in treated ducks, which refer to hepatic inflammation.

In control ducks, the inflammatory proteins parameters were stabilized. By contrast, in treated ducks, all the inflammatory proteins levels, such as: alpha 1 globulins, alpha 2 globulins, beta globulins and A/G ratio were increased over time during the study, except serum albumin, which was nearly constant. Moreover, significant elevation was observed in those parameters when compared treated ducks at the 8th hr with the 21st day post-dosing. Also significant elevation was recorded in alpha 1 globulins at days 3 and 7, alpha 2 globulins at days 14 and 21, beta globulins and A/G ratio from day 7 until the end of the experiment. By contrast in turkeys, serum protein electrophoresis analysis was nearly constant during the experiment in both groups (control and treated), except for a slightly significant increase which was recorded in alpha 2 globulins at day 3, and in A/G ratio at 8th hr post-dosing, (table 33).

Table 33: Effects of FBs on serum protein electrophoresis

FB1+FB2 mg/kg.BW	Duck		Turkey	
	0	10	0	10
Albumine (g/l)				
0	17.76 ± 0.5	17.78 ± 1.1	17.42 ± 0.6	18.08 ± 0.9
3	16.66 ± 2.5	18.5 ± 2.2	14.06 ± 1.3	16.44 ± 1.3
7	16.52 ± 1.6	16.66 ± 0.8	14.76 ± 0.4	14.44 ± 1.3
14	15.9 ± 1.6	18.4 ± 2.2	15.72 ± 1.4	14.42 ± 1.1
21	15.46 ± 1.3	14.77 ± 3.2	15.86 ± 1.2	15.38 ± 0.9
Alpha-1 globulin (g/l)				
0	1.74 ± 0.4	1.5 ± 0.3	2.84 ± 0.3	2.65 ± 0.3
3	1.82 ± 0.4	2.66 ± 0.6 *	2.36 ± 0.4	2.34 ± 0.2
7	1.28 ± 0.3	2.26 ± 0.5 *	2.16 ± 0.5	3.02 ± 0.8
14	1.72 ± 0.5	2.78 ± 2.5	2.32 ± 0.6	2.42 ± 0.3
21	1.96 ± 0.4	3.37 ± 1.9	2.82 ± 0.1	2.88 ± 0.4
Alpha-2 globulin (g/l)				
0	6.84 ± 0.6	6.9 ± 0.9	5.72 ± 0.3	5.7 ± 0.5
3	7.46 ± 1.8	8.14 ± 1.2	4.82 ± 0.4	5.54 ± 0.3 *
7	8.66 ± 0.8	9.38 ± 0.7	6.2 ± 0.8	7.44 ± 4.8
14	7.9 ± 0.9	9.66 ± 1.3 *	6.06 ± 0.9	5.96 ± 0.3
21	6.96 ± 0.6	9.41 ± 2 *	6.28 ± 0.5	6 ± 0.2
Beta globulin (g/l)				
0	8.64 ± 0.5	9.08 ± 0.5	10.18 ± 0.9	9.83 ± 0.3
3	8.86 ± 2	10.38 ± 1.2	9.02 ± 2.3	9.86 ± 0.7
7	8.3 ± 1.4	11.96 ± 2 *	9.78 ± 2.4	10.12 ± 2.6
14	8.32 ± 0.9	12.24 ± 0.8 *	9 ± 1	8.32 ± 0.4
21	8.32 ± 0.9	11.72 ± 3.4 *	8.74 ± 0.4	9.24 ± 0.8
Gamma globulin (g/l)				
0	1.28 ± 0.4	1.28 ± 0.2	4.24 ± 0.9	3.78 ± 0.4
3	1.82 ± 0.5	2.36 ± 0.7	3.78 ± 1.1	4.22 ± 1
7	1.8 ± 0.3	2.6 ± 0.3	6.06 ± 1.8	5.8 ± 1.5
14	1.94 ± 0.4	1.92 ± 0.2	4.48 ± 0.9	4.48 ± 1.2
21	1.74 ± 0.3	2.21 ± 0.6	3.46 ± 0.2	3.5 ± 0.7
Albumin to globulins A/G				
0	0.96 ± 0.1	0.95 ± 0.1	0.76 ± 0.04	0.82 ± 0.04 *
3	0.84 ± 0.1	0.79 ± 0.04	0.71 ± 0.1	0.75 ± 0.1
7	0.83 ± 0.04	0.64 ± 0.04 *	0.68 ± 0.1	0.56 ± 0.1
14	0.8 ± 0.1	0.69 ± 0.1 *	0.73 ± 0.1	0.68 ± 0.1
21	0.81 ± 0.04	0.58 ± 0.2 *	0.74 ± 0.1	0.71 ± 0.1

Values were obtained from five birds per group (four birds on day 21 for ducks that received FBs) 8 hr after the first administration of FBs (day 0) and at days 3, 7, 14, and 21 and expressed as mean ± SD.*Two-way ANOVA was done to compare treated and control groups by species (significantly different, P < 0.05)

CHAPTER 4: DISCUSSION

In the literature review part it has been demonstrated that, ducks are more sensitive to FBs toxicity than turkeys, whereas, the accumulation of FB1 and Sa in tissues are higher in turkeys than in ducks. The objective of the PHD was to investigate the causes which lead to different toxicity between ducks and turkeys to FBs exposure. Two experiments were conducted on ducks and turkeys under the same conditions in order to explain their different toxicity to FBs exposure. i) Toxicokinetics of fumonisin B2 experiment was conducted to investigate if the absorption and/or eliminations of FB2 are different between the two species. ii) FBs-toxicity experiment was investigated, if the FBs have different toxicity effects between ducks and turkeys in several aspects: general toxicity (mortality, body and organs weight, and hepatotoxicity parameters), amount of free sphingolipids in tissue and serum, correlation between accumulation of free sphingolipids in liver and hepatotoxicity, amount of sphingolipid phosphorylated forms in liver and serum, correlation between amount of sphingolipid phosphorylated form in liver and hepatotoxicity, correlation between accumulation of free sphingolipids and sphingolipid phosphorylated forms in liver, correlation between amount of FBs and free sphingolipids, oxidative damages, and serum inflammatory proteins.

I. FB2-Toxicokinetic Experimental

Toxicokinetics of FBs, such as FB2 and FB3 are poorly documented when compared to FB1. The toxicokinetics of FB2 had only been studied in rat and non-human primates [28-51], whereas toxicokinetics of FB1 had been investigated in several animal species, including avian species [23-24-30-31-32-34-35-55]. In parallel, FB2 has similar structure to FB1 [18-21], and it has similar toxic and carcinogenic effects to FB1 in rodents [48]. Hence, the results obtained here in this study were compared with available data derived from FB2 and FB1 by using different doses, in different animal species.

The method of analysis used for the quantification of FB2 in plasma is similar to the described method for FB1 analysis in serum and tissues of ducks and turkeys [23-24-280]. The results obtained by this method were within the same ranges of those obtained by using SAX column for the extraction of FBs from plasma of rat, ducks and turkeys [23-24-28-58-280].

Strong linearity of the whole method validation (with $R^2 = 0.994$) was obtained from fortified plasma with standards 0.025 to 0.250 $\mu\text{gFB2/ml}$. The limit of detection (LOD) and quantification (LOQ) were estimated at around 0.01 and 0.025 $\mu\text{g FB2/ml}$ of plasma, respectively. These results agreed with the results obtained from rat plasma fortified with FB2, in which the LOD obtained by using SAX extraction was 0.02 $\mu\text{g FB2/ml}$ [28]. Also, they agreed with data obtained from ducks and turkeys, in which the LOQ obtained by using SAX extraction for serum fortified with FB1 was 0.025 $\mu\text{g FB1/ml}$ [23-24]. The mean recovery rate of FB2 from fortified plasma for each level of contamination was 63% \pm 5. This recovery rate obtained was lower than the only one already described by Shephard for FB2 in rat plasma (80.4% \pm 4.5) [58]. By contrast, it was close to the recovery rate of FB1 in the plasma of ducks and turkeys (60%) [23-24].

The elimination of FB2 from plasma after intravenous dosing in ducks and turkeys was fitted according to a bi-exponential equation, as reported in monkeys injected with FB2 [51], and rats, monkeys, layer hens, and ducks injected with FB1 [23-30-32-55].

Distribution phase ($T_{1/2 \alpha}$) of FB2 in ducks and turkeys (3.8 and 1 min, respectively) was rapid as the $T_{1/2 \alpha}$ obtained for FB1 in ducks, turkeys, layer hens and pigs (2.6, 1.7, 2.5 and 2.2 min, respectively) [23-24-30-31].

The elimination half life ($T_{1/2 \beta}$) was slower in ducks than in turkeys (32 and 12.4 min, respectively). However, the elimination half life of FB2 in both species was at the same range with data only available on monkeys (18 min) by using quite similar dose to our study (2 mg FB2/kg b.w.) [51]. These results support and explain the prolonged quantification of FB2 in duck serum when compared to turkey serum, after IV injection (two and one hours after dosing, respectively). Also, these results reveal the most important persistence of FB2 in duck tissues than in turkey ones. On the other hand, in ducks, the elimination half life of FB2 (32 min) was quite similar to the one obtained for FB1 (26.2 min), whereas, the MRT of FB2 (12.9 min) was 2 times lower than FB1 (24.2 min), (table 34) [23]. In turkeys, the elimination half life ($T_{1/2 \beta}$) and the MRT of FB2 (12.4 and 5 min, respectively) were 5 times lower than the one obtained for FB1 (85.5 and 25 min, respectively), (table 34) [24]. These results suggest that FB2 persistence in tissues is less pronounced than FB1.

The clearance of FB2 was similar in ducks and turkeys (9.3 and 8.7 ml/min/kg, respectively). By contrast, the clearance of FB1 was higher in ducks than in turkeys (19.3 and 8.7 ml/min/kg, respectively), (table 34) [23-24]. Interestingly, in turkeys the same value of clearance was recorded with FB2 and FB1 (8.7 ml/min/kg). By contrast, the clearance of FB2 in ducks was lower than FB1 (9.3 and 19.3 ml/min/kg, respectively). The differences between clearances of FB2 and FB1 in ducks are probably due to incorrect estimation of clearance of FB2, because, FB2 and FB1 have similar chemical structure [18-21], and the experimental dose of FB2 was small (1 mg/kg b.w.).

Table 34: Toxicokinetic parameters of FBs after IV dosing in ducks and turkeys

Parameters	FB2 (1 mg/kg .b.w.)		FB1 (10 mg/kg .b.w.) [23-24]	
	Duck	Turkey	Duck	Turkey
T _{1/2α} (min)	3.8	1	2.6	1.7
T _{1/2β} (min)	32	12.4	26.2	21.3
MRT (min)	12.9	5	24	25
Cl (ml/min/kg)	9.3	8.7	19.3	8.7

T_{1/2α}: distribution half-life; T_{1/2β}: elimination half-life; MRT: mean residence time; Cl: total plasma clearance

The bioavailability of FB2 was very low in ducks and turkeys. This result was estimated by lack of detection of FB2 in plasma after oral dosing in both species, except two cases of turkeys when toxin level was higher than LOQ, and lower than 50 ng/ml. These results agreed with previous data obtained in rats and monkeys by using the same method and near dose of our experimental (7.5 mg FB2/kg b.w.) [28-51]. No detection of FB2 in plasma was reported in rats (as in ducks) [28], whereas, trace amount of FB2 (20-40 ng/ml) was recorded in the plasma of monkeys (as in turkeys) [51]. On the other hand, the bioavailability of FB2 is less pronounced than that of FB1 in all tested animal species. Bioavailability of FB1 in ducks, turkeys, rat and monkeys were 2.3, 3.2, 3.5 and 2 %, respectively [23-24-32-35]. Interestingly, the bioavailability of FB1 and FB2 are more pronounced in turkeys than in ducks.

Apparently, the toxicokinetic parameters of FB2 do not differentiate greatly between ducks and turkeys. In addition, the absorption of FB2 is lower than FB1 in those species. Thence, the toxicokinetics of FB2 could not explain the different

toxicity between those species to FBs exposure. Furthermore, the risk of toxicity by FB2 is less pronounced than FB1 in those species.

II.FBs-Toxicity Experimental

All previous researches which studied the FBs toxicity in ducks and turkeys were conducted by different protocols (bird age, tested dose, duration of experiment, and route of administration), which made it very difficult to get precise comparative details between those works. Therefore, the present study investigated the causes of different toxicity between ducks and turkeys to FBs exposure by using the same time and conditions.

The results obtained in this study were discussed with previous data reported in avian and /or other animal species. Also, the results were discussed with fumonisin mechanism of action, and new hypotheses of different toxicity between ducks and turkeys to FBs exposure were proposed.

1. General toxicity and serum biochemistry

The mortal effect of FBs was observed in ducks, but not in turkeys. These results were in agreement with previous data. The FBs was able to induce mortality at low dose in ducks fed 20 mg FB1/kg of feed per day, by force feeding over 12 days [158], and at high dose in young broiler fed 125 mg FB1/kg of diet for 3 days [147]. By contrast, mortal effect of FBs was not reported in turkeys exposed to low dose of 20 mg FB1/kg of feed, for long period 63 days [156], or high dose of 475 mg FB1 /kg of diet for short period of 21 days [154], and in laying hens fed high dose of 200 mg FB1/kg of diet for long period 14 months [155]. Those results demonstrated that turkeys were more resistant to FBs exposure than ducks, and that ducks showed the highest sensitivity to FBs exposure among avian species.

Decrease of feed intake and body weight gain were only observed in ducks, and not in turkeys. Decrease of feed intake and body weight were commonly observed in poultry after exposure to FBs, but they were dose-dependent. For example, they were reported in ducks and broilers which had consumed contaminated diet at a level higher than 30 mg FB1/kg for few weeks and in turkeys fed high dose of 75 mg FB1/kg for 3 weeks [150-154-165]. In our study, the decrease

of body weight gain in ducks was not linked to malabsorption of feed, because feed conversion ratio was constant during the experimental. However, it was linked to the decrease of feed intake. In parallel, the decrease of feed intake in ducks was not linked to the bad flavor of feed, because exposition of the toxin was conducted by force feeding. It was a consequence of FBs toxicity. Therefore, those results showed better tolerance to FBs exposure in turkeys than in ducks.

FBs increased liver weight in ducks, whereas FBs increased gizzard weight in turkeys. These results agree with previous studies. Heavy liver was recorded in ducks fed low dose of 32 mg FB1/kg for 77 days [165], in turkeys fed mild dose of 75 mg FB1/kg for 21 days [149-154], and broiler which consumed high dose of 450 mg FB1/kg for 21 days [151]. Also, increased gizzard weight was noticed in ducks fed high dose of 128 mg FB1/kg for 77 days [165], and in turkeys fed high dose of 100 mg FB1/kg for 21 days [310]. The main causes of heavy liver are: i) hepatic steatosis which is considered a normal physiological condition in migrating birds, geese and ducks [158-302-311], and pathological condition in non-migrating birds including geese, ducks and turkeys [223-302-306]. ii) Cell proliferation, strong hepatocellular hyperplasia was observed in ducks receiving 5 mg FB1/kg .b.w. by daily oral administration over 12 days [163], and in turkeys which consumed contaminated diet at a level of 75 mg FB1/kg for 21 days [149-154]. iii) Inflammatory cases, which led to escape of intravascular fluid and blood cells from blood vessels to interstitial tissue. Inflammatory infiltrations was reported in ducks receiving 5 mg FB1/kg .b.w., by daily oral administration over 12 days [163], and in ducks consuming contaminated diets higher than 30 mg FB1/kg of feed, for one week [170-171]. Unfortunately, histological examination was not conducted in our study. However, heavy duck livers in the present study could be attributed to liver inflammation.

The serum biochemistry analyses were investigated to confirm the different toxicity between ducks and turkeys to FBs exposure.

Hyperproteinemia was only observed in ducks, but not turkeys. These results were in agreement with previous data. Hyperproteinemia was obtained in ducks receiving 5 mg FB1/kg .b.w., by daily oral administration over 12 days [163], and in ducks fed 128 mg FB1/kg of diet for 7 days [165-170-171]. While, concentration of total serum proteins was constant in turkeys fed low dose of FBs 20 mg FB1+FB2/kg

of feed, over long a period of 9 weeks [156], in turkeys which consumed mild dose of 50 mg FB1/kg of diet for long period 3 months [153], in turkeys which consumed high dose of 200 mg FB1/kg of diet for short time 21 days [310], and broiler fed high dose 400 mg FB1/kg of diet for 21 days [148]. The hyperproteinemia is commonly observed in dehydration cases [219], but in our experiment, ducks were not dehydrated. On the other hand, hyperproteinemia was also combined to inflammatory processes, as it will be discussed later in section- 4 (inflammatory response).

Hypercholesterolemia was only observed in ducks, while cholesterol remained constant during the study in turkeys according with previous data. Hypercholesterolemia was recorded in ducks fed more than 30 mg FB1/kg of diet for 7 days [165-170-171], and in broilers which consumed high contaminated diets at level 400 mg FB1/kg of feed for 21 days [148]. Whereas, serum cholesterol was not affected in turkeys fed mild dose of 50 mg/kg of diet for a long period (more than 9 weeks) [153-156], laying hens consumed high contaminated diet of 200 mg FB1/kg of feed for a long period of 420 days [155], broilers were fed mild dose of 80 mg FB1/kg feed for 21 days [146]. Hypercholesterolemia has been reported during acute hepatic steatosis in ducks [158-302-311]. However, post-mortem examination of ducks did not show any signs of hepatic steatosis such as, an increase in liver size and a change of liver color. Additionally, it has been demonstrated that disturbance in sphingolipids metabolism leads to disturbance in lipids metabolism pathways including cholesterol, and inducing hypercholesterolemia [190-214-250-312]. This hypothesis showed that ducks are more sensitive to disturbance of sphingolipids metabolism than turkeys, as discussed later in section- 2 (Sphingolipids alterations).

ALT remained nearly constant during the study in both species. This result agreed with data obtained in ducks fed dose 8 mg FB1/kg of diet for a long period of 77 days [165], turkeys fed low dose of 20 mg of FB1+FB2/kg of feed over a long period of 9 weeks [156], turkeys which consumed high dose 250 mg FB1/kg of diet for a short period of 21 days [149], and laying hens fed 200 mgFB1/kg of diet for a long period of 420 days [155]. By contrast elevation of ALT was detected in other animal species, such as pigs exposed to single oral dose of 5 mg FB1/kg b.w. [63], ponies fed contaminated diets at level 44 mg FB1/kg of feed, for 10 days [252], calves

which consumed 148 mg FBs/kg of feed, for 10 days [133]. The insensitivity of ALT to FBs exposure in avian species is maybe linked to the lower concentration of ALT in their cytoplasm [246-247-294-295], when compared to mammals.

In both species, AST was less sensitive to FBs exposure, which increased latter after several days post-dosing. However, AST was more sensitive to FBs exposure in turkeys (appeared at day 14 of treatment) than ducks (appeared at day 21 of treatment). The lower sensitivity of AST to FBs exposure in ducks and turkeys agreed with previous data obtained in avian species, in which AST was only elevated at high doses of FBs exposure. The elevation of AST was reported in broilers fed contaminated diets with 80 mg FB1/kg of feed for 21 days [146], turkeys fed high dose of 100 mg FB1/kg of diet for 21 days [310], laying hens supplied with high dose of 200 mg FB1/kg of diet for a long period of 112 days [155]. Whereas, insensitivity of AST was demonstrated in ducks which consumed high dose of 128 mg FB1/kg of diet for 77 days [165], turkeys fed with 20 mg of FB1+FB2/kg of feed over long a period of 9 weeks [156], and laying hens which consumed contaminated diet with 100 mg FB1/kg of diet for a long period of 420 days [155]. The lower sensitivity of AST to FBs exposure in avian species is maybe related to AST position inside the cell (20% in cytoplasm and 80% in mitochondria) [245-308]. On the other hand, the different sensitivity of AST between ducks and turkeys to FBs exposure is probably correlated to higher concentration of AST in turkeys (control and treated groups) when compared to ducks.

LDH increased rapidly after 3 days of treatment until the end of the study in ducks, While LDH was only observed after 7 days of exposure in turkeys. The faster raise of LDH after FBs exposure in ducks when compared to turkeys was in agreement with previous data. The elevation of LDH was recorded after 7 days of feeding ducks with 32 mg FB1/kg [165]. By contrast, LDH was not affected in turkeys which consumed 20 mg FB1+ FB2/kg of diet for 63 days [156], and in broilers fed 400 mg FB1/kg of diet for 21 days [148]. The common causes which lead to an increase in LDH are: hepatic cells damages, muscular disorder and atrophy and hemolysis [246-247-294-295-308]. Additionally, muscular disorder disease and muscular atrophy cases were combined with a decrease in body weight [246-294-295], which was not reported during our study in both species. Also, sample hemolysis which leads to

false elevation of LDH was not observed during the experimental manipulation. Hence, elevation of LDH in ducks was an indication of hepatic damage.

Obviously, the serum biochemistry analysis confirms the different toxicity between ducks and turkeys to FBs exposure, as it shows marked elevation of hepatotoxicity parameters in ducks when compared to turkeys.

2. Sphingolipids alterations

2.1. Free sphingolipids

2.1.1. Free sphingolipids in tissues

In both species, Sa in liver and kidneys showed high sensitivity to FBs exposure, which elevated rapidly after 8 hr post-toxication. This result agreed with previous studies, which reported an increase of Sa and Sa:So ratio in tissues in ducks fed very small dose of 2 mg FB1/kg feed, for 7 days [165-170-171], in turkeys which consumed maximum level of fumonisins in avian feed recommended by the European Union (20 mg/kg of feed), for 7 days [156], and broiler fed with 25 mg FB1/kg of diet, for 42 days [153]. This study confirms rapid sensitivity of sphingolipids metabolism to FBs exposure in both species.

In both species, the disturbance of sphingolipids metabolism by FBs exposure was more pronounced in the kidneys than in the liver, in agreement with data obtained with ducks fed 2 mg FB1/kg of feed, for one week [165-170-171], Sprague-Dawley rats which consumed 15 mg FB1/kg of feed, for 4 weeks [264], and F344 rats fed 5 mg FB1/kg of feed, for 6 weeks [264]. By contrast, the liver and kidneys had the same sensitivity to fumonisins exposure in turkeys fed 20 mg FB1+FB2/kg of feed, for one week [156]. The different sensitivity between liver and kidneys to FBs in both species could be attributed to different sensitivity of cell death and cell generation programs in those tissues [171].

Interestingly, the accumulation of Sa in tissues is higher in turkeys than in ducks, whereas, signs of FBs toxicity (mortality, decreased body weight gain and hepatotoxicity parameters) were more pronounced in ducks than in turkeys. This result agreed with previous researches which demonstrated higher sensitivity of ducks to FB1 toxicity than turkeys, as written above [149-154-156-158-165].

Surprisingly, accumulation of Sa in tissues reached the maximum level faster in turkeys (3 days post-dosing) than in ducks (7 days post-dosing). This result was confirmed by the slope of sphinganine accumulation curve in tissues, which were stronger in turkeys (≈ 16) than in ducks (≈ 2.5). Indeed, the slope of sphinganine accumulation curve in liver was quite similar to the slope of sphinganine accumulation curve in kidneys in ducks and turkeys. Those results revealed that the accumulation capacity of Sa in both species was not tissue-dependence (liver and kidney). However, it was dependent on the differences between avian species. Furthermore, the slope of sphinganine accumulation curve was in accordance with rapid accumulation of Sa in turkey tissues compared to duck tissues. Unfortunately, those results are difficult to compare with other data, because, all previous research protocols measured the disturbance of sphingolipids metabolism in tissues after several days post FBs exposure.

2.1.2. Free sphingolipids in serum

The amount of Sa in serum was higher in ducks than in turkeys. In addition, the escape of Sa from tissues to serum was faster in ducks than in turkeys, which appeared after 8 hr and 3 days post dosing, respectively. These results show higher sensitivity of ducks to FBs exposure than turkeys, in agreement with previous data. The elevation of Sa in serum was observed in ducks which had consumed very low dose of 2 mg FB1/kg feed, for 7 days [165-170-171], and in turkeys and broilers fed mild contaminated diet of more than 70 mg FB1/kg of feed for 21 days [146-154]. Whereas, elevation of Sa and Sa:So in the serum was not detected in turkeys which had received mild dose of 50 mg FB1 /kg of feed, for a long period (3 months) [153], and broilers which had consumed 40 mg FB1 /kg of feed, for 21 days [146].

Interestingly, the liberation ability of Sa was quite similar in treated ducks and turkeys (5 and 3 times higher than control groups, respectively). Consequently, the liberation ability of Sa from tissue to serum is unable to explain the different toxicity between ducks and turkeys to FBs exposure.

2.1.3. Correlations between Sa in liver, Sa in serum and hepatotoxicity parameters

In both species, sphinganine in tissues and serum was rapidly increased after 8 hr post-dosing. Except in turkey serum, where it increased after 3 days post-dosing. By contrast, hepatotoxicity parameters were elevated later after 3 days post-dosing in ducks, and they were not affected in turkeys. Those results agreed with previous researches, which demonstrated higher sensitivity of sphingolipids metabolism to FBs exposure than hepatotoxicity parameters. Lowest-observed-effect-level (LOEL) to increase Sa and Sa:So ratio in liver was 2 and 20 mg FB1/kg feed, for 7 days in ducks and turkeys, respectively [156-165-170-171], and 20 mg FB1/kg feed, for 21 days in broilers [146], whereas Lowest-observed-effect-level (LOEL) to increase hepatotoxicity parameters was 32 mg FB1/kg feed, for 7 days in ducks [165-170-171], and more than 80 mg FB1/kg feed, for 21 days in turkeys and broilers [146-148-149-310]. Thence, the present study confirms that disturbance of sphingolipids metabolism is the best biomarker to FBs exposure when compared with other toxicity parameters in both species.

Additionally, the accumulation of Sa in duck liver was significantly correlated with hepatotoxicity parameters, such as protein and LDH. By contrast, no correlation was obtained between accumulation of Sa in turkey liver and hepatotoxicity parameters. These results agreed with previous data. Strong correlation between accumulation of Sa in liver and hepatotoxicity parameters was reported in ducks which received a dose of 5 mg FB1/kg b.w via oral administration over 8 days [164], and in ducks fed a dose higher than 30 mg FB1/kg of feed for 7 days [170]. By contrast, accumulation of Sa in liver, without any increase of hepatotoxicity parameters were observed in turkeys fed maximum level of fumonisins in avian feed recommended by the European Union (20 mg FB1+FB2/kg of feed), for 63 days [156]. Also, strong correlation between accumulation of Sa in liver and hepatotoxicity parameters was observed in other species, such as rats fed contaminated diet at level 88.6 mg FB1/kg of feed for 10 days [26]. This result demonstrates that turkeys are more tolerant than ducks to accumulation of Sa in liver. Subsequently, it is suggested that accumulations of free sphingolipids in tissues are not responsible for different

toxicity to FBs exposure between ducks and turkeys. This suggestion agreed with previous data obtained in mice, which demonstrated a significant decrease of free sphingolipids (Sa and So) without a significant decrease in hepatotoxicity in mice treated with fumonisin plus myriocin when compared to a group treated with fumonisin alone [87]. By contrast, a significant increase of free sphingolipids (Sa and So) without a significant increase in hepatotoxicity was observed in mice treated with FB1 plus silymarin when compared to the group treated with FB1 alone [236]. Concurrently, that suggestion was in opposition with the rat study, which demonstrated the responsibility of free sphingolipids to induce hepatotoxicity [26]. In fact, the rat study was not as precise as our study. That is because the rat study was not a comparative study between high and low sensitive rat strains to FBs exposure, but it was only conducted on highly sensitive rat strains to FBs exposure. By contrast, our study was a comparative experiment between high and low sensitive avian species (ducks and turkeys) to FBs exposure.

In both species, the accumulation of Sa in liver was correlated with hypercholesterolemia. Similar result was demonstrated in ducks which consumed a contaminated diet at a dose higher than 30 mg FB1/kg of feed for 7 days [170], and in ducks which received 5 mg FB1/kg, b.w. orally, for 8 days [164]. Moreover, the correlation between accumulation of Sa in liver and hypercholesterolemia was reported in other species, such as rats fed 71 mg FB1/kg of feed for 4 weeks [313]. This phenomenon could be linked to the ability of accumulate free sphingolipids inside the cells to disturb lipid metabolism pathways (including cholesterol metabolism) by activating certain lipid metabolism enzymes, such as phospholipase D, and inhibiting others (monoacylglycerol acyltransferase, and protein kinase C) [190-214-250-312].

For both species, the amount of Sa in serum was in a significant correlation with the amount of Sa in liver. This result showed that the amount of Sa in serum is strongly dependent on the amount of Sa in liver in ducks and turkeys. On the other hand, the equations of correlation coefficient of Sa were different between ducks and turkeys ($R^2 = 0.6370$ and 0.7201 , respectively). These differences in the equations reveal to higher rate of Sa escape from liver to serum in ducks compared with turkeys, which explains the higher amount, and quickly elevation of Sa in duck

serum when compared with the turkeys. Therefore, those results are probably indicative of duck hepatocyte damage.

Actually, all investigations on free sphingolipids failed to explain the different toxicity between ducks and turkeys to FBs exposure. Thence, it was interesting to study the impact of FBs on cell sphingolipids phosphorylation mechanism in both species in order to explain their different toxicity to FBs exposure.

2.2. Sphingolipids phosphorylated forms (liver and serum)

In the literature review part it was demonstrated that a portion of the accumulated free sphingolipids in the cells are metabolized into sphingolipid-1-phosphate (Sa/So1P) and then cleaved into a fatty aldehyde and ethanolamine phosphate [79-180-181-188]. In parallel, Sa1P and So1P act as a promoting factor for cell proliferation, cell growth, anti-inflammatory and anti-apoptosis [178-179-201-235]. Therefore, the catabolism of free sphingolipids (phosphorylation) is considered a cell protective mechanism against accumulation of free sphingolipids, which was studied in ducks and turkeys as way to answer their different toxicity to FBs exposure.

In both species, elevation of Sa1P in liver was rapidly detected after 8 hr post-dosing. This result agreed with previous data. Elevation of Sa1P in liver was observed in ducks after consuming a high dose of more than 30 mg FB1/kg of feed, for 7 days [171]. Also, implication of FBs in the increase of sphingolipid phosphorylated forms has been reported in different animal species such as mice injected via IP route with 20 mg FB1/kg.b.w/day, for 2 days [208], piglets fed contaminated diet with 30 mg FB1/kg of feed for 42 days [102], and in cell culture [312]. Unexpectedly, the phosphorylation ability of Sa in liver was quite similar in ducks and turkeys (8 and 6 times higher than control groups, respectively). This result demonstrated that cell catabolism of free sphingolipids (phosphorylation) are not responsible for different toxicity between ducks and turkeys to FBs exposure. However, the slope of Sa1P accumulation curve was stronger in treated turkeys than in treated ducks (177 and 36, respectively). This result is in accordance with rapid elevation of Sa1P in turkey liver than in duck liver, which reached maximum levels

after 3 and 7 days, respectively. These results also reveal to high phosphorylation capacity of Sa in turkeys than in ducks.

In both species, the elevation of Sa1P in serum appeared later after 3 days post-dosing. Interestingly, the liberation ability of Sa1P in ducks and turkeys was quite similar (4 and 3 times higher than control, respectively). But, the average amount of Sa1P in serum was 1.5 times higher in ducks than in turkeys. Unfortunately, no information about Sa1P in avian serum is available for comparison with our results. The higher amount of Sa1P in duck serum compared to turkey serum is may be due to higher rate of Sa1P escape from liver to serum in ducks, which reveals hepatocyte damage in ducks. This suggestion is supported by the results obtained in ducks during the present study, which demonstrated an elevation of serum protein, cholesterol, LDH, and Sa at same time with Sa1P, after 3 days post-dosing.

In both species, very strong significant correlation was obtained between the amount of Sa1P and Sa in liver, and the equations of correlation were very similar (ducks $R^2 = 0.8813$, and turkeys $R^2 = 0.8856$) (P value < 0.001). This result has been recorded in ducks fed more than 30 mg/kg of feed, for one week [171]. The result shows that the amount of Sa1P is strongly dependent on the amount of Sa in liver in both species. Furthermore, no correlations were observed between the accumulation of Sa1P in liver and hepatotoxicity parameters in both species.

Therefore, those results suggest that the phosphorylated forms of sphingolipids are not responsible for different toxicity between ducks and turkeys to FBs exposure.

2.3. Correlation between amount of FBs and Sa in liver

In the present study, the amount of FBs in liver was higher in turkeys than in ducks. This result agreed with prior results obtained from toxicokinetics studies of FB1 in these species, in which the absorption of FB1 was higher in turkeys than in ducks (F (%) = 3.2 and 2.3, respectively), and the excretion of FB1 was lower in turkeys than in ducks (CL (ml/min/kg) = 7.5, and 19.3, respectively) [23-24]. Also, it supports our toxicokinetic study of FB2 in ducks and turkeys, which recorded slightly higher absorption of FB2 in turkeys than in ducks.

On the other hand, significant correlation was recorded between the accumulation of Sa and FBs in the livers of ducks and turkeys ($R^2 = 0.7801$ and 0.7128 , respectively). However, the equations of correlation were different between ducks and turkeys. This result has been reported for the first time in avian species. Furthermore, strong correlation between accumulation of FB1 and Sa in liver was observed in other animal species such as rodents (for example: rats which had consumed contaminated diet at level 88.6 mg FB1/kg of feed for 10 days [26]). Those results show that the amount of Sa in the liver is strongly dependent on the amount of FBs in the liver of ducks and turkeys. This correlation was linked to the ability of fumonisin to interfere with sphingolipids biosynthesis by blocking ceramide synthase enzyme [187-190-191-192-197-214-215].

Eventually, the accumulation of Sa in liver was more pronounced in turkeys than in ducks. Whereas, the amount of Sa in serum was higher in ducks than turkeys. In addition, signs of toxicity were clear in ducks than turkeys, and hepatotoxicity parameters were correlated with the amount of Sa in the liver of ducks, but not in turkeys. Therefore, it was suggested that accumulations of free sphingolipids in liver are not responsible for different toxicity between ducks and turkeys to FBs exposure. Furthermore, elevation of Sa in serum is a consequence of FBs exposure, which reveals hepatocyte damage in ducks.

Accumulation of Sa1P in liver was more pronounced in turkeys than in ducks, whereas, phosphorylation ability of Sa was quite similar in both ducks and turkeys. In parallel, the amount of Sa1P in liver was strongly dependent on the amount of Sa in liver in both species. Moreover, the accumulation of Sa1P in liver was not correlated to hepatotoxicity parameters in both species. Thence, the phosphorylation mechanism of free sphingolipids and accumulation of Sa1P in liver are insufficient to explain the different toxicity between ducks and turkeys to FBs exposure. On the other hand, a higher amount of Sa1P in duck serum compared to turkey serum is probably an indication of hepatocyte damage in ducks.

Interestingly, in both species, the amount of Sa in liver was dependent on the amount of FBs.

Finally, it appears that disturbance sphingolipids metabolism by fumonisins are not responsible for different toxicity between ducks and turkeys to fumonisins

exposure. Also, it is probable that fumonisins have other mechanisms to induce hepatic toxicity, such as oxidative damage effects and/or inflammatory response.

3. Oxidative damages

In this study, FBs did not induce significant changes in oxidative damage parameters, such as CAT and GSH, which tested in both species. Unfortunately, the oxidative damage effects of FBs are poorly documented in poultry. However, FBs inducing hepatotoxicity by their oxidative damage effects have been observed in few studies in vitro and vivo at high doses, such as in rats fed high dose of 250 mg FB1/kg diet for 21 days [314], rats fed 0.08 – 0.16 mg FB1/100g b.w/day for 2 years [45], rats injected IP with 0.5mg FB1/kg b.w/day for 7 days [315], and also in cell culture [316]. Failure of FBs at low dose (as used in our study) to increase oxidative damages parameters was reported in ducks which received 45 mg FB1/kg b.w. by daily oral administration over 12 days [163], and other animal species, such as rats which consumed contaminated diet at level of 10 mg FB1/kg diet for 21 days [314].

Additionally, the results obtained in the present study were supported by recent results obtained in our lab after feeding ducks, turkeys and broilers with same dose and period used in our experimental (10 mg FB1/kg diet for 21 days). The recent lab results demonstrated absent effects of FBs on several oxidative damage parameters, such as SOD, CAT, GSH peroxydase, GS reductase, GSH, GSSG, and MAD (data not shown).

It is clearly that oxidative damage effect of FBs failed to explain the different toxicity between ducks and turkeys to FBs exposure.

4. Inflammatory response

Fumonisins increased inflammatory proteins only in ducks, but not turkeys. The increase of alpha-1 and 2 globulins, beta globulins, gamma globulins, and A/G ratio were more pronounced when comparing ducks at the first day to the last days of treatment, rather than when comparing treated and control groups at the day of exposure. The separation of alpha-1 on alpha-2 globulins was difficult. Thence, its values were not precise. The inflammatory response of FBs was evaluated by its

effect on increase of beta globulins, gamma globulins, and A/G ratio. The elevation of A/G ratio in ducks was a consequence of elevation of globulins, but not albumin. These results agreed with the results obtained in this study and with previous researches, which demonstrated the ability of FBs to induce hyperproteinemia in ducks, but not in turkeys as discussed above in section-1 (General toxicity and serum biochemistry). Hyperproteinemia without clear histological signs of hepatic inflammation was observed in ducks exposed to 5 mg FB1/kg .b.w. by daily oral administration over 12 days [163], and in ducks fed with contaminated feed at doses higher than 30 mg FB1/kg of feed, for 7 days [170-171]. For this reason, Serum Protein Electrophoresis was studied for the first time in both species after FBs exposure.

Concurrently, elevation of alpha and gamma globulins were observed in acute and chronic inflammatory response cases due to infectious diseases, nephritis, and hepatitis [319-320-321]. Therefore, the elevations of inflammatory proteins in our study are probably indicator of duck hepatotoxicity, as post-mortem examination of ducks did not reveal infection or nephritis. Also, elevation of beta globulins was used as an indicator for hyper lipoproteinemia, in particular hypercholesterolemia [319-320]. For that reason, an increase of beta globulins in ducks, but not in turkeys supported and confirmed our hypercholesterolemia results, which was reported only in ducks, but not in turkeys, as discussed above in section-1 (General toxicity and serum biochemistry).

On the other hand, FBs inducing hepatotoxicity by its impact on inflammatory cells (Kupffer cells) has been reported in rodents [166-233-238]. For example, elevation of hepatotoxicity enzymes (ALT and AST) were obtained in mice treated with FB1 alone. Meanwhile, a significant diminishment in hepatotoxicity enzymes (ALT and AST) was observed in mice treated with mixture of fumonisin and gadolinium (complete elimination of Kupffer cells) [238].

Apparently, the different inflammatory response between ducks and turkeys, and role of Kupffer cells to induce hepatotoxicity could explain the different toxicity between those species to FBs exposure.

III. Conclusion

The objective of this work was to investigate several hypotheses that could explain differences of FBs toxicity between ducks and turkeys.

Toxicokinetic evaluation of FB2 was investigated because previous studies have suggested that parameters of absorption and elimination of this mycotoxin could be different from FB1. The results obtained here reveal only weak differences between these two species. Additionally, the values obtained from toxicokinetics studies of FB2 and FB1 were at the same range, except for the absorption of FB2 which was found to be lower than FB1 in both species.

The FBs-toxicity experiment confirms higher sensitivity of ducks than turkeys to FBs exposure and demonstrates that:

- The increase of sphingolipids contents in tissue and serum is rapid and precede signs of toxicity in both species, which confirmed that sphingolipids parameters are the best biomarkers to FBs exposure.
- Accumulation of Sa in liver is correlated with toxicity in ducks and not in turkeys whereas the amount of Sa in liver was higher in turkeys than in ducks.
- Accumulation of Sa1P in liver is strongly correlated with the amount of Sa, the Sa1P to Sa ratio is the same in the two species, so, the phosphorylation rate fails to explain the difference of toxicity.
- The amount of Sa in liver is correlated with the amount of FBs in ducks and turkeys, the amount of FB1 being more important in turkeys than in ducks.
- FBs had no significant effect on oxidative damage parameters whereas preliminary investigations on inflammatory response revealed the ability of FBs to increase inflammatory proteins in ducks, but not in turkeys.

IV. Prospective studies

Although phosphorylation of Sa into Sa1P failed to explain the differences of toxicity of FBs between ducks and turkeys, it cannot be excluded that differences of sphingolipids metabolism between the two species could be related to differences of toxicity. Indeed several metabolites of Sa are formed in cells, some of them being cytotoxics, others being protective [68-174-188-192-323]. Thence, it would be of interest to study the effect of FBs on ceramide metabolism in ducks and turkeys.

The FBs-toxicity study revealed the ability of FBs to increase inflammatory proteins in ducks but not in turkeys. This result agrees with literature data demonstrating hyperproteinemia in ducks, but not in turkeys [153-156-163-165-170-171-310]. Although the mechanism of this increase remain uncertain, it can be pointed out that an effect of FBs on cells involved in the immune response has been reported as a key mechanism of toxicity in rodents [166-233-238]. Therefore, a specific effect of FBs on Kupffer cells may induce hepatotoxicity in ducks but not in turkeys. This hypothesis is under investigation, as the effect of mycotoxins at low doses of exposure in the avian species.

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